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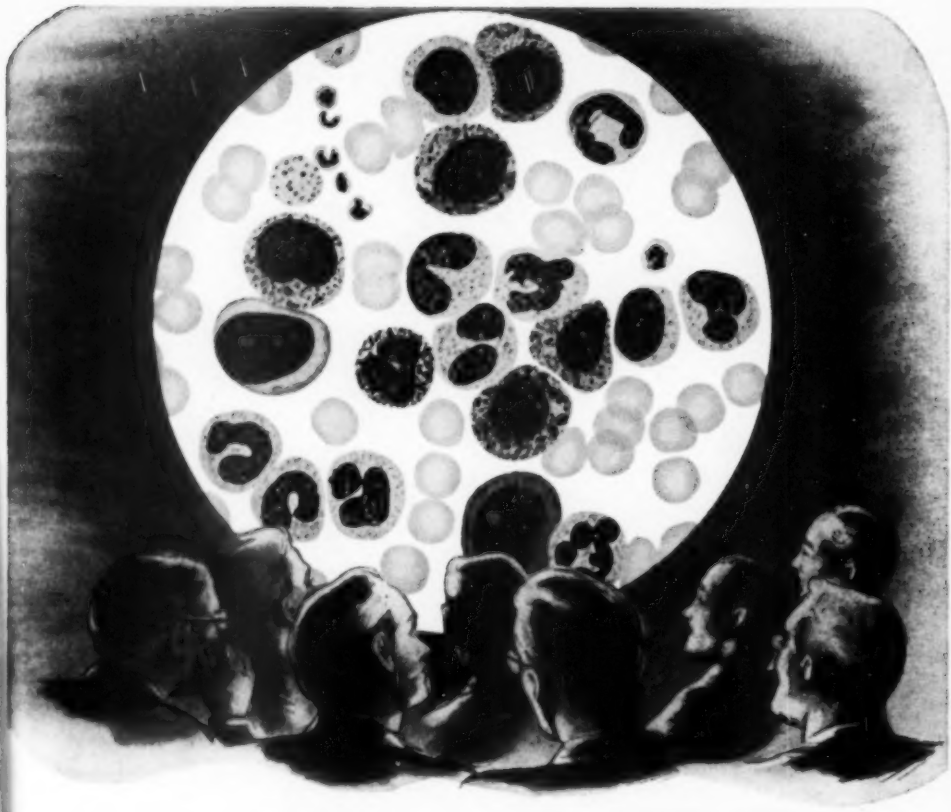
TABLE OF CONTENTS FIRST PAGE



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CONTENTS

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*Bronstein, M. R., and Witkind, E.: Am. J. Med. Sci. *222*, 677, Dec., 1951.

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ARCHIVES OF PATHOLOGY

VOLUME 54 JULY 1952 NUMBER 1

ENDOCHONDRAL BONE GROWTH IN THE CHICK

S. BURT WOLBACH, M.D.
AND
D. MARK HEGSTED, Ph.D.
BOSTON

ENDOCHONDRAL bone growth in the chick is sufficiently different from that in mammals to make a somewhat detailed description desirable as a preliminary to the recording of effects experimentally produced upon it.

The most recent accounts of the resorption of cartilage in the development of the long bones of birds are those of Brachet (1893), Lubosch (1923-1924), and Fell (1925). Brachet's [1] studies extended through the period of the 3d to 19th day of incubation and were not sufficiently detailed for our purposes. Lubosch [2] established the fact that by the 14th day after hatching the formation of the marrow cavity is complete, and that the zones of endochondral bone growth at the ends of the long bones are established in final form for the remainder of the growth period. He gave scant but succinct descriptions of histological sequences and stated clearly that the cartilage processes upon which bone is deposited contain cells, whereas in mammals they consist only of cartilage matrix.

Fell [3] did not describe the long bones beyond one day after hatching, at which time there are cones of cartilage extending from the ends of the bones into the incompletely formed marrow cavity. She described the limited endochondral ossification present at this stage and recorded in detail the sequences in the early development of long bones attending the disappearance of cartilage in advance of penetrating blood vessels.

> . . . It would appear that most if not all the elements of the marrow exert a chondrolytic action. It is possible that the multinucleated giant cells may have a destructive effect upon the cartilage but in the earlier stages of the formation of the marrow these structures are not sufficiently numerous to constitute the only, or indeed, the chief agents of erosion and the excavations at this period usually contain only blood vessels, connective tissue and osteoblasts. In some areas the tissue forming the walls of the blood vessels appears to be chiefly involved in the resorption of the cartilage . . . .

This study was aided by research grants from The Nutrition Foundation, the Williams-Waterman Fund for the Combat of Dietary Disease, and Swift and Company, Chicago.

From the Division of Nutritional Research, Division of Laboratories and Research, The Children's Hospital; the Department of Nutrition, Harvard School of Public Health, and the Department of Biological Chemistry, Harvard Medical School, Boston.

1. Brachet, A.: Étude sur la résorption du cartilage et le développement des os longs chez les oiseaux, Internat. Monatsschr. f. Anat. u. Physiol. **10**:391, 1893.

2. Lubosch, W.: Die Bildung des Markknochens beim Hühnchen und bei Sängetieren und das Wesen des endochondralen Ossifikation in historischer Betrachtung, Morphol. Jahrb. **53**:49, 1923-1924.

3. Fell, H. B.: The Histogenesis of Cartilage and Bone in the Long Bones of the Embryonic Fowl, J. Morphol. & Physiol. **40**:417, 1925.

Because the chick bones studied were those of controls in experiments upon chicks kept upon abnormal dietary regimens from two to three days after hatching to 23 days of age, our studies were made at ages of 19 to 23 days, according to the duration of the experiments.

The regions selected for the study of growth sequences in normal and experimental chicks were the distal end of the femur, proximal end of the tibiotarsus, lumbar and sacral vertebrae, and the base of the skull.

THE DIET OF CONTROL CHICKS

The composition of the diet fed the control chicks was as follows:

Sucrose	51.0 gm.
Vitamin-free casein	18.0 gm.
Corn oil	5.0 gm.
Gelatin	10.0 gm.
Salts IV*	5.0 gm.
$CaHPO_4$	1.0 gm.
Brewer's yeast	10.0 gm.
Choline chloride	0.3 gm.
Thiamine hydrochloride	400.0 γ
Riboflavin	800.0 γ
Pyridoxine hydrochloride	400.0 γ
Nicotinic acid	3,000.0 γ
Calcium pantothenate	1,500.0 γ
Viosterol	2,000.0 I. U.

In addition, 100 γ of 2-methyl-1,4-naphthoquinone (Menadione U. S. P.), 100 γ of alpha-tocopherol in corn oil, and 1 drop of haliver oil (approximately 5,000 I. U. in vitamin A) were fed twice a week to each chick by medicine dropper.

* Hegsted, D. M.; Mills, R. C.; Elvehjem, C. A., and Hart, E. B.: J. Biol. Chem. 138:459, 1941.

Normal Chicks Used for Histological Study

No.	Source of Vitamin A in Diet	Other Additions	Weight, Gm. 15 Days	16 Days	19 Days	Age Killed, Days	Final Weight, Gm.
414	Haliver oil	1.5% liver extract	...	110	140	22	164
430	Haliver oil	1.5% liver extract	...	115	148	22	182
431	Haliver oil	.....	...	121	...	18	148
460*	1% cod liver oil added	.....	...	110	130	32	213
457	1% cod liver oil added	.....	...	127	150	32	252
366	2% cod liver oil added	5% liver extract	97	...	...	32	246
357	2% cod liver oil added	5% liver extract	105	...	...	32	233
360	2% cod liver oil added	5% liver extract	119	...	...	32	255
362	2% cod liver oil added	5% liver extract	122	...	...	32	252
186	Kept on commercial chick ration	.....	113	...	...	26	218

* This chick had diarrhea, 14th to 17th days of age.

All chicks fed this diet were killed between the 18th and 22d day of age, inclusive. Other chicks were fed the diet with haliver oil omitted and 1% cod liver oil added, and some were kept on a commercial chick food.

The chicks killed on the 32d day of age were kept on slightly different rations in that haliver oil was omitted and additions of 2% cod liver oil and 5% liver extract were made.

ENDOCHONDRAL BONE GROWTH IN LONG BONES [4]

The growth of the long bones in all birds [2] presents differences from that in mammals.

In both birds and mammals the first bone formed is of periosteal origin. In the chick, as soon as shell of bone is formed in the midshaft region, the enclosed carti-

4. All descriptions are made from bones fixed in 10% formalin (4% formaldehyde solution), embedded in celloidin, and stained with hematoxylin and eosin.

lage, after the blood vessels have entered from the periosteum, is excavated without being replaced by trabecular bone, unlike that in the mammal. Growth at the ends is wholly cartilaginous. As the cartilage core is undergoing destruction, the only ossification in the shaft is at the periphery of the cartilage, where the latter becomes tunneled by blood vessels. At hatching, cones of cartilage are present at the ends of the bones. Concurrent erosion of the cartilage and tunneling by blood vessels result, on about the 14th day, in the establishment of the counterpart of the epiphyseal cartilage of mammals. No separate center of ossification forms at the epiphysis. During the growth period a zone of hyaline cartilage is present between the epiphyseal cartilage and the articular cartilage. At the age period 18 to 32 days, with which the present study is concerned, we can distinguish three zones, articular, hyaline, and epiphyseal or growth cartilage (Figs. 1 and 2).

Fig. 1.—Longitudinal section through the distal end of the femur of an 18-day-old normal chick (No. 431). At the upper right the articular cartilage is shown. Note the vascularization of the cartilaginous epiphysis and the absence of resorption where blood vessels pass through the proliferative zone of the epiphyseal cartilage. Reduced from 20.8 diameters.

The articular cartilage is a relatively thin stratum of flattened cells having an eosinophilic matrix. Adjacent to attachments of the joint capsule and the ligaments of the knee joint it merges into fibrocartilage. Deep to the surface of the joint it merges into the hyaline cartilage through a succession of cells of increasing size. Although the transition into cartilage with isogenous grouping of cells is rather abrupt, there is no definite line of demarcation between the articular cartilage and the zone of hyaline cartilage which may be regarded as a cartilaginous epiphysis.

The zone of hyaline cartilage is characterized by a basic-staining matrix and isogenous groups of cells with thin but definite "capsules." It is penetrated by blood vessels which extend into it from the epiphyseal cartilage and which branch and

anastomose with one another. It is clearly demarcated from the epiphyseal cartilage, chiefly because of the deeper staining of the cells and the matrix of the latter. When stained with thionin blue after formalin fixation, the matrix of the cartilaginous epiphysis is colored clear blue in sharp contrast to that of the epiphyseal cartilage, which is colored bluish purple. In adult chickens this cartilaginous epiphysis is

Fig. 2.—Higher power magnification of a part of Figure 1. Note the loss of the columnar arrangement of cartilage cells in the zones of growth and maturation, the loops of advancing blood vessels, and the lateral excavations of the tunnels. × 50.

absent. Presumably, the blood vessels and surrounding cells which we have described are, at a later period of growth, concerned in its removal. Adjacent to the epiphyseal cartilage the cells of the cartilaginous epiphysis become more and more flattened and in longitudinal sections of the long bones appear as narrow spindle-shaped cells, placed singly and in end-to-end pairs. In sections transverse to the

long axis of the bone these cells appear in various discoid forms, hemicircular, crescentic, and triangular.

The blood vessels of the cartilaginous epiphysis are surrounded by small, undifferentiated cells similar in appearance to cells surrounding the advancing tips of the blood vessels tunneling the epiphyseal cartilage. The blood vessels include small arteries, veins, and capillaries; the last, where exclusively present, may be tortuous and show anastomoses between adjacent loops. Whether the vessels entering from the shaft of the bone communicate with blood vessels from the perichondrium has not been positively determined; but, because in cross sections at the level where blood vessels from the epiphyseal cartilage enter the cartilaginous epiphysis no blood vessels with a media could be found, the arteries and the veins in the cartilaginous epiphysis must have origin elsewhere.

The zone of proliferating cells of the epiphyseal cartilage is very wide as compared with that of mammalian bones. The cells are extremely flattened, closely packed, crescentic, or wavy as seen in longitudinal sections. In general, the crescentic shape prevails, with the concavity toward the diaphysis. In sections transverse to the long axis of bones the cells appear discoid with irregular outlines. Mitotic figures are present throughout the considerable width of the proliferating zone, which presumably ends on the diaphyseal side, where the cells have become distinctly larger, with plump, ovoid nuclei, and surrounded by an increased amount of matrix. In the epiphyseal border of the zone of proliferation the cells are packed in columns narrowly separated from one another, but this arrangement is wholly lost where the cells have reached sufficient size to be globular in shape. There is presented to the blood vessels advancing from the diaphysis a zone of enlarged (hypertrophic) cells without arrangement in columns and equally embedded on all sides with matrix, in striking contrast to the columns of maturing and matured cells in the mammalian epiphyseal cartilage.

Vascular penetration of the epiphyseal cartilage in chicks (and in all birds and cold-blooded vertebrates [2]), while functionally similar, is quite different from that in mammals in pattern and in important details. The cytological sequences of the cartilage from the discoid cell of the zone of proliferation to the mature cell are essentially similar to those in the mammal except that (in the chick) degeneration is less complete preparatory to ossification sequences. Shrunken but viable-appearing nuclei, surrounded by fragments of cytoplasm, persist until the capsule has been eroded through, both in advance of and lateral to the blood vessels tunneling the epiphyseal cartilage.

The pattern of vascular tunneling of the epiphyseal cartilage of the chick is very different from that of mammals. In the latter, the epiphyseal cartilage on the diaphyseal side is composed of cells in linear arrangement, each row or column one cell in thickness and surrounded by calcified matrix of considerable thickness, while the matrix between individual cells of the columns is very much thinner. Each column, following degeneration of the presenting cartilage cell, is penetrated by capillary loops accompanied by osteoblasts, resulting in tunnels whose cross-sectional dimensions are those of mature cartilage cells (Fig. 3). The walls of the tunnels consist of calcified acellular matrix upon which osteoblasts deposit bone. The tunnels persist as such only for a short distance toward the diaphysis, often not exceeding in length the width of the epiphyseal cartilage, but varying as the growth rate of the bone varies under experimental conditions. Bone is deposited on the calcified

cartilage of the tunnels, which are soon destroyed by partial or complete osteoclastic resorption, which may vary in rate and extent according to experimentally produced conditions. Left behind is the familiar structure of cancellous bone of the metaphyseal region. Cartilage matrix incorporated in the deposit of bone persists for a long time, particularly where, as a result of remodeling, trabeculae of endochondral bone growth become surrounded in the shaft by appositional bone of endosteal and

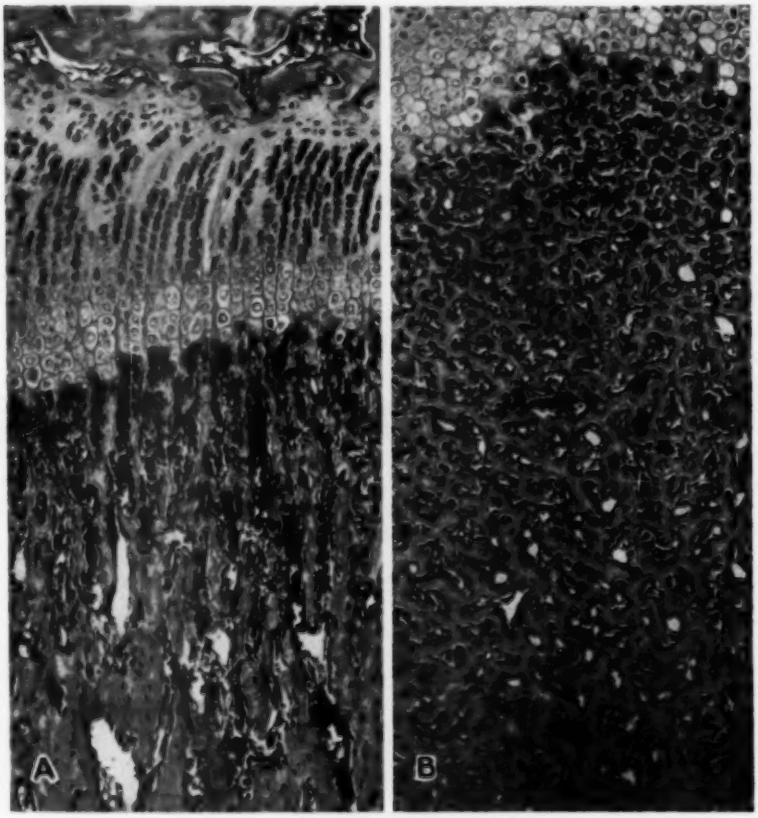

Fig. 3.—*A*, longitudinal section of the distal femoral epiphyseal cartilage of a young rat. × 120.

B, cross section of the distal femoral epiphyseal cartilage of a young rat. At the upper end, because of the convexity of the condylar surface, "cleared" cartilage cells are seen as well as the earliest penetration by blood vessels. × 120.

periosteal origin. It becomes less and less in amount as the bone grows. The mechanism of its final disappearance is unknown.

In the chick the tunneling of the mature cartilage is widely and fairly uniformly spaced (Figs. 1 and 4). Longitudinal sections and transverse sections at various levels through the epiphyseal cartilage of normal chicks 18 to 32 days of age show

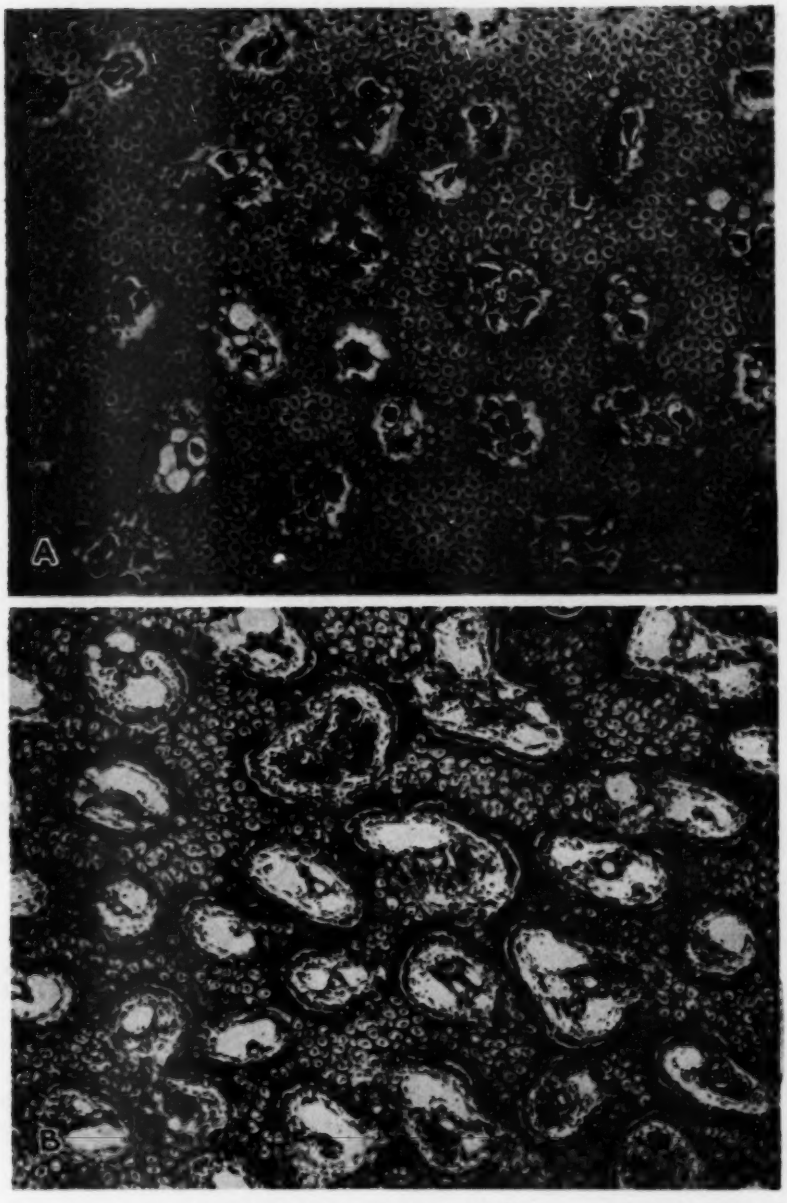

Fig. 4.—*A*, cross section of the epiphyseal cartilage of a 22-day-old normal chick (No. 414), to show early tunneling before osteoid deposition has occurred. Note the number of blood vessels in each tunnel and the disappearance of cartilage cell capsules on the sides adjacent to the tunnels. Note absence of chondroclasts and osteoblasts. × 120.

B, section from the same block from which *A* was obtained, to illustrate a later stage of tunneling. Note the early stages of the lateral resorption of the cartilage walls, the presence of osteoid deposits, and rows of osteoblasts and multinucleated giant cells. × 120.

only slight variations in individual chicks. At the levels of most advanced penetration of the epiphyseal cartilage the spacing of the tunnels during the 18- to 32-day period, measured between centers, varies from 0.27 to 0.35 mm. Vessels from the tunnels that have passed through the proliferative zone into the cartilaginous epiphysis are also fairly evenly distributed, and in the latter structure are 0.44 to 0.6 mm. apart from one another.

The fact that the spacing of the tunneling of the epiphyseal cartilage has notable uniformity and constancy implies the presence of a determining factor. No morphological indication of such in normal chicks can be seen in the zone of mature cartilage cells in advance of the invading blood vessels. The advancing blood vessels at levels where erosion of cartilage predominates over osteogenesis consist of thin-walled tubes without media. They give off branches which freely anastomose with one another. The extreme tip is a loop (Fig. 2). In transverse sections through the van of vessel penetration, two to seven or more vessels of capillary dimensions appear in cross section. Often the cross sections are elliptical and crescentic in shape, indicative of tortuosity and anastomoses (Fig. 4*A*).

Blood vessels with a smooth muscle media are present only at the level where bone deposition is abundant.

The blood vessels, even at the very tips, are surrounded by cells. Those at the tips of advancing blood vessels are closely packed together and often lie enclosed by the terminal capillary loop. They are two to three times the size of the red blood corpuscles, are oat-shaped, and presumably are undifferentiated cells. Diaphysealward the accompanying cells become more and more numerous. Multinucleated giant cells—chondroclasts—are abundant wherever cartilage is being resorbed. An occasional one may be at the tip of an invading vessel. Other cells, in order of frequency in progression diaphysealward, are elongated connective-tissue-like cells, osteoblasts, and marrow cells of all types.

The multinucleated giant cells—chondroclasts—are identical in appearance with the multinucleated giant cells—osteoclasts—present in the same bones in regions of bone resorption. They become more numerous in the cartilage tunnels in the direction toward the diaphysis, presumably in relation to increasing degrees of calcification of the cartilage matrix. While undoubtedly operative in the opening of cartilage cells and in removal of bone deposited on the tunnel walls, their numbers do not seem sufficient for regarding them as the whole or major agent in the first tunneling of the epiphyseal cartilage. Multinucleated giant cells may not be present or identifiable in foci where cartilage cell capsules have been opened. Instead there are groups of osteoblasts. The presence of these cells, surrounded by osteoid matrix, within the capsules, may explain the conviction of some authors that cartilage cells can transform to osteoblasts. Cartilage cells in the immediate proximity of advancing blood vessels are abruptly changed to dimly outlined structures, with and without nuclear remains and without capsules on the side bordering the blood vessels. The content of the cells appears to be replaced by a faintly staining homogeneous material suggestive of an origin from greatly swollen disappearing capsules or matrix (Fig. 4*A*).

Multinucleated giant cells are usually not present at the extreme tip of vascular advancement. Cells of all sorts may be few, and the process seems to be one of chondrolysis wherever blood-containing capillaries come into close proximity to

mature epiphyseal cartilage cells. The caliber of the tunnels increases by a similar process for a short distance but increase of caliber becomes restricted and modified as soon as osteoblasts become applied to exposed cartilage matrix. After bone matrix deposition has been established, the cartilage between adjacent tunnels becomes irregularly removed by osteoclasis, and communications are established. The caliber of these passages is at first approximately that of the tunnels they connect, but as growth progresses they increase in size concurrently with the resorption of the walls of the tunnels. The continuation of this process, seen in cross sections, results in a labyrinth of tortuous canals with cartilaginous walls of irregular thick-

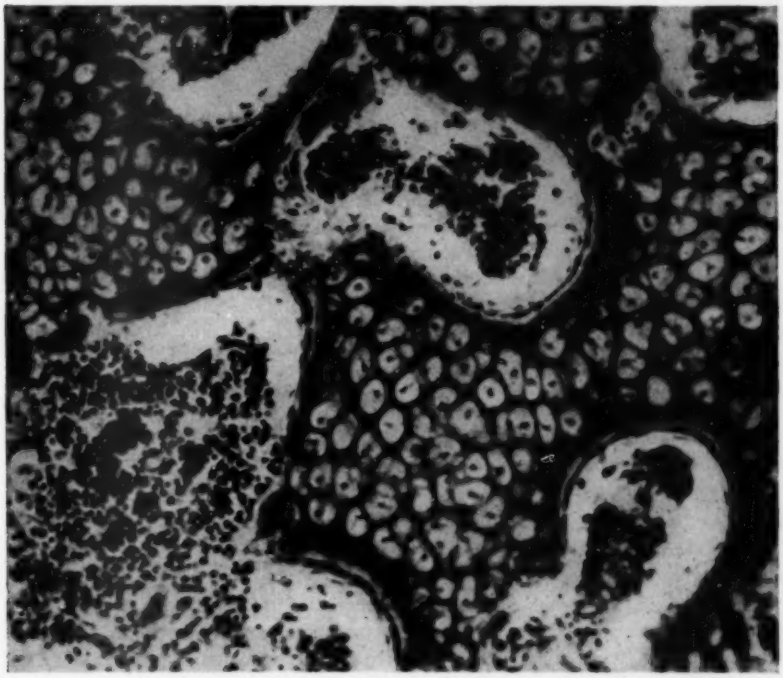

Fig. 5.—Detail of a cross section of epiphyseal cartilage of an 18-day-old chick. This chick, for the last three days, was fed a reduced amount of food, but the slowed growth sequences are wholly normal in character. Note the shrunken nuclei of mature cartilage cells and the details mentioned for Figure 4*B*. × 300.

ness, in places undergoing removal by chondroclasis, in places becoming coated with bone (Figs. 4*B* and 5).

When seen in longitudinal sections the remains of the walls of the tunnels appear as columns of cartilage cells, sometimes enclosed by layers of bone on both sides, often covered by bone on one side and undergoing chondroclastic resorption on the other. Diaphysealward the "columns" become thinner and more and more broken up by chondroclasis and osteoclasis until they disappear in the diaphyseal marrow.

In contrast to analogous sequences of growth in mammals is the survival, in the chick, of apparently viable cartilage cells in slender "columns" upon which bone has been deposited and far distant from their origin in epiphyseal cartilage. Because in the remodeling attending linear growth of long bones bone of endochondral origin becomes incorporated into cortical bone, cartilage cells may be found here in regions where all other bone of endochondral origin has disappeared. This corresponds to the persistence of cartilage matrix in a similar location in growing mammalian bones. In the chick the final vestige of cartilage appears as a basic-staining irregular tracery of remnants of the cell capsules. The manner in which cartilage cells disappear and the source of the osteoblasts which replace them in regions where they are apparently completely enclosed by bone present a problem not yet satisfactorily solved.

ENDOCHONDRAL BONE GROWTH IN THE AXIAL SKELETON

In the age period covered by this study, endochondral ossification is active in the vertebrae. We have employed serial sectioning of a few vertebrae in cross sections of the seventh thoracic vertebra and in longitudinal sections, one series inclusive of thoracic 7 to lumbosacral 12; another series inclusive of thoracic 6 to lumbosacral 10. The seventh thoracic vertebra, the 14 lumbosacral vertebrae, and the first coccygeal vertebra form a unit structure, the synsacrum, in which the spinal canal is greatly enlarged (lumbosacral vertebrae 3 to 10) to accommodate the egg-shaped glycogen body, a structure peculiar to birds, intimately connected with the spinal cord and traversed by the central canal and separating the dorsal funiculi to form the so-called sinus rhomboidalis.[5]

There are no joints between the vertebrae forming the synsacrum,[6] but there is epiphyseal cartilage at both ends of each vertebra; the vertebrae are separated from one another by hyaline cartilage. In the vertebrae of the chick, as in the long bones, there are no epiphyseal centers of ossification.

In sections of vertebrae, seven regions of endochondral ossification were studied, including the dorsal spine, the two lateral processes, the two of the body providing for increase in cross sectional diameters, and the ones at the anterior and posterior ends providing for linear growth.

In the skull, in frontal plane sections, endochondral ossification was observed at the articulations of the sphenoid and temporal bones, the articulations of the temporal and occipital bones, and several regions in the temporal bone complex not precisely anatomically identified.

In every essential detail, endochondral bone growth in the vertebrae and in the skull, in the chick, is identical with that at the ends of long bones and presents the same contrasts to endochondral bone growth in the same locations in mammals as do the growth processes at the ends of the long bones.

In the growth of all bones studied, epiphyseal cartilages increase in width by appositional growth from the perichondrium. The shapes of bones are preserved

5. Watterson, R. L.: Development of the Glycogen Body of the Chick Spinal Cord, J. Morphol. **85**:337, 1949.

6. Kaupp, B. F.: Anatomy of the Domestic Fowl, Philadelphia and London, W. B. Saunders Company, 1918. Chamberlain, F. W.: Atlas of Avian Anatomy, East Lansing, Mich., Michigan State College Agricultural Experiment Station, 1943.

during growth as in mammals by continuous remodeling that involves resorption of bone and new bone formation from endosteal and periosteal sources.

COMMENT

Skeletal growth in the chick, as in mammals, includes three processes: (1) growth of epiphyseal cartilage and its replacement by bone, (2) appositional growth of bone and cartilage, and (3) remodeling involving both resorption and new formation of bone. One and (3) are interdependent to a marked degree.

A detailed understanding of the morphological sequences of all three is essential for the recording and appraisal of effects produced upon skeletal growth by deficient diets or by substances added in excess of physiological needs.

The only age differences in the period we have covered (18 to 32 days after hatching) are a moderate decrease of the length of the cartilage tunnels with age and a diminution of the width of the zone of growing cells (zone of increase in size of cells) of the epiphyseal cartilage. These differences are negligible for the purpose of comparisons of the normal and experimentally produced modifications of growth.

Bone growth, until completed, is in all details a continuation of fetal growth and follows a pattern involving responses of competent tissues to organizing agents. With this in mind we have noted, without discussion, the passage of blood vessels, quite regularly distributed, through the epiphyseal cartilage into the cartilaginous epiphysis, unaccompanied by effect upon the zones of growth and proliferation. These blood vessels are apparently not concerned in the sequences of endochondral bone growth during the age period studied, although they arise from vessels situated in the top of the tunnels.

SUMMARY

Endochondral bone growth in the chick proceeds by widely spaced tunneling of epiphyseal cartilage by blood vessels and accompanying cells from the diaphyseal side. The epiphyseal cartilage, in contrast to that of mammals, does not present a columnar arrangement of matured or degenerated cartilage cells to the advancing blood vessels. Degeneration or clearing of cartilage cells is not complete as in mammals, and no differences are apparent between cartilage cells in immediate proximity to advancing blood vessels and those situated lateral to the paths to be followed by penetrating blood vessels.

In the tunneling process of the epiphyseal cartilage at the tips of advancing blood vessels, the matrix (including capsules of cells) disappears and the cells, though in part surrounded by capsules, degenerate and disappear. The sequences are similar to those described by Fell [3] in the early ossification of the chick's skeleton, and attributed to chondrolysis, a characterization with which we are forced to agree.

Multinucleated giant cells may or may not be present at the advancing tips of the tunnels; they become numerous in older regions of the tunnels—presumably because of greater degrees of calcification of the cartilage matrix.

Ossification proceeds by the depositing of bone upon the walls of the tunnels. This is not a symmetrical process, because soon after formation lateral resorption of cartilage accompanied by chondroclasts takes place at apparently irregularly distributed areas in the length of the tunnels and establishes communications between adjacent tunnels. At this stage, multinucleated giant cells—chondroclasts—are almost invariably present and seem to be the chief agent in the removal of the

cartilage. In older regions of the tunnels, bone, as well as cartilage, is removed and a greater complexity of cartilage columns, wholly or in part surrounded by bone, gives rise to an appearance of trabecular bone extending to the level of nearly completed ossification. The prevailing architecture, as seen in longitudinal sections, is that of parallel trabeculae in which apparently viable cartilage cells persist. In corresponding locations in mammalian bone growth, only remnants of calcified cartilage matrix are found. The manner of tunneling and the long duration of cartilage that is in process of concurrent ossification and resorption give rise to a metaphyseal region very much greater in extent than that found in mammalian bone growth.

The histological preparations were made by Mr. John J. Burke, department technician. The photomicrographs were made by Mr. John Carabitses, of the Department of Pathology, The Children's Hospital.

VITAMIN A DEFICIENCY IN THE CHICK

Skeletal Growth and the Central Nervous System

S. BURT WOLBACH, M.D.
AND
D. MARK HEGSTED, Ph.D.
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IN MANY mammalian species (rat, mouse, guinea pig, dog, ferret, calf) vitamin A deficiency has a profound effect upon the growth of bones, productive of pressure upon the nervous system.[1] Wolbach and Bessey [1a] proved that in the rat and the guinea pig skeletal growth was severely retarded before growth of soft tissues, including the nervous system, was impaired, so that the compression of the contents of the cranium and the spinal canal was solely responsible for paralyses. Wolbach,[1c, d] categorically stated that the retardation of skeletal growth was primarily that of endochondral bone growth accompanied by failure of the remodeling sequences which are essential for normal growth patterns of bone. Less growth of bone, abnormal in pattern because of continuation of appositional bone deposition, and retardation or failure of compact bone formation were other important features stressed by Wolbach.[1c, d]

Mellanby [1e] attributed the pressure upon the nervous system in dogs to overgrowth of bone, explainable by a preponderance of osteoblastic over osteoclastic activity. In his book [1e] he gives his first and only attention to epiphyseal cartilage responses to vitamin A deficiency, cursorily and disparagingly.

The purposes of this report are to document statements made by one of us [1c, d] in regard to the effects of vitamin A deficiency upon the skeletal growth of the chick, and to record the important and constant results obtained in a relatively small series of chicks at a time when attention is being paid to the skeletal and nervous system responses to vitamin A deficiency in another avian species.[2]

This study was aided by research grants from The Nutrition Foundation, the Williams-Waterman Fund for the Combat of Dietary Disease, and Swift and Company, Chicago.

From the Division of Nutritional Research, Division of Laboratories and Research of The Children's Hospital; the Department of Nutrition, Harvard School of Public Health, and the Department of Biological Chemistry, Harvard Medical School, Boston.

1. (*a*) Wolbach, S. B., and Bessey, O. A.: Vitamin A: Deficiency and the Nervous System, Arch. Path. **32**:689, 1941; (*b*) Tissue Changes in Vitamin Deficiency, Physiol. Rev. **22**:233, 1942. (*c*) Wolbach, S. B.: Vitamin A Deficiency in Relation to Skeletal Growth, Proc. Inst. Med. Chicago **16**:116, 1946; (*d*) J. Bone & Joint Surg. **29**:171, 1947. (*e*) Mellanby, E.: A Story of Nutritional Research: The Effects of Some Dietary Factors on Bones and the Nervous System, Baltimore, Williams & Wilkins Company, 1950, Chap. 6 and 8; (*f*) Chap. 7.

2. (*a*) Fletcher, D. E., and Rigdon, R. H.: Neurologic Manifestations Associated with Vitamin A Deficiency in Young Ducks, Arch. Neurol. & Psychiat. **61**:199, 1949. (*b*) Neurologic Manifestations Associated with Vitamin A Deficieicy in Young Ducks, editorial, Nutrition Rev. **7**:188, 1949. (*c*) Rigdon, R. H.; Rude, J. C., and Bierie, J. G.: Effect of Hypervitaminosis A and Hypovitaminosis A on the Skeleton of a Duck, A. M. A. Arch. Path. **52**:299, 1951. (*d*) Rigdon, R. H.: Pathologic Lesions in the Nervous System of the Duck Fed a Ration Deficient in Vitamin A, ibid. **53**:239, 1952.

EXPERIMENTAL STUDY

The diet, minus sources of vitamin A, was that described in the preceding paper on "Endochondral Bone Growth in the Chick." [3] The chicks were given the diet as soon as received from the hatchery at 1 or 2 days of age. Only those used for histological bone studies are listed in the accompanying table. Some of these and a few not listed were also used for gross dissections, after decalcification, of the cranium and portions of the spinal column.

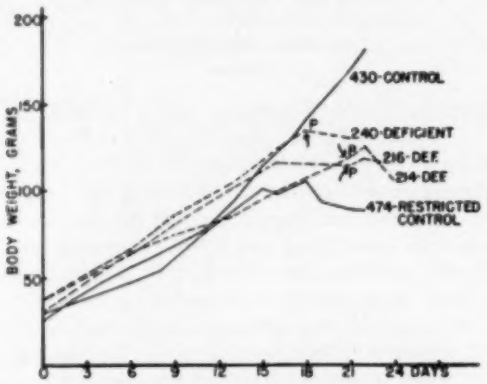

Fig. 1.—Weight graphs of three vitamin-A-deficient chicks, one normal control, and one fed a reduced amount of a normal diet. The arrows indicate the day on which paralyses were noted.

Data on Normal Chicks Used for Histological Study

Chick	Paralyses		Maximum Weight		Death		Comment
	Day	Weight, Gm.	Day	Weight, Gm.	Day	Weight, Gm.	
240	17	124	..	...	20	131	Died
229	19	146	20	148*	28	118	Killed
216	21	119	21	119	23	117	Died
214	21	130	22	136*	24	107	Died
236	25	131	24	136*	28	114	Killed
271	16	74	15	77	19	...	Died
473*	15	105	14	109	19	81	Force-fed last 4 days; killed
481*	15	114	15	114	21	81	Force-fed last 3 days; died
385*	15	112	21	139	21	139	Killed†
255	22	158	25	158*	27	147	Force-fed last 5 days; killed
267	24	162	25	168*	27	152	Force-fed last 3 days; killed

* These chicks received 5% instead of 10% yeast in the diet, and 1.5% liver extract was added.
† The gain in weight occurred during the last four days and was probably due to an error in feeding. The bones were typical of vitamin A deficiency.

All of the vitamin-A-deficient chicks were paralyzed before death, a few as early as the 15th day but usually not before the 20th day. In most instances weight gain was satisfactory until paralyses were noted and occasionally for one or two days following signs of paralyses. The weight graphs (Fig. 1) include those of three representative A-deficient chicks, and the days on which paralyses (*P*) were noted are indicated. The relation of onset of paralyses to gain in weight shows that in chicks, as well as in rats,[1c,d] the soft tissues continue to grow at an approximately normal rate after severe retardation of skeletal growth has occurred.

3. Wolbach, S. B., and Hegsted, D. M.: Endochondral Bone Growth in the Chick, Arch. Path., this issue, p. 1.

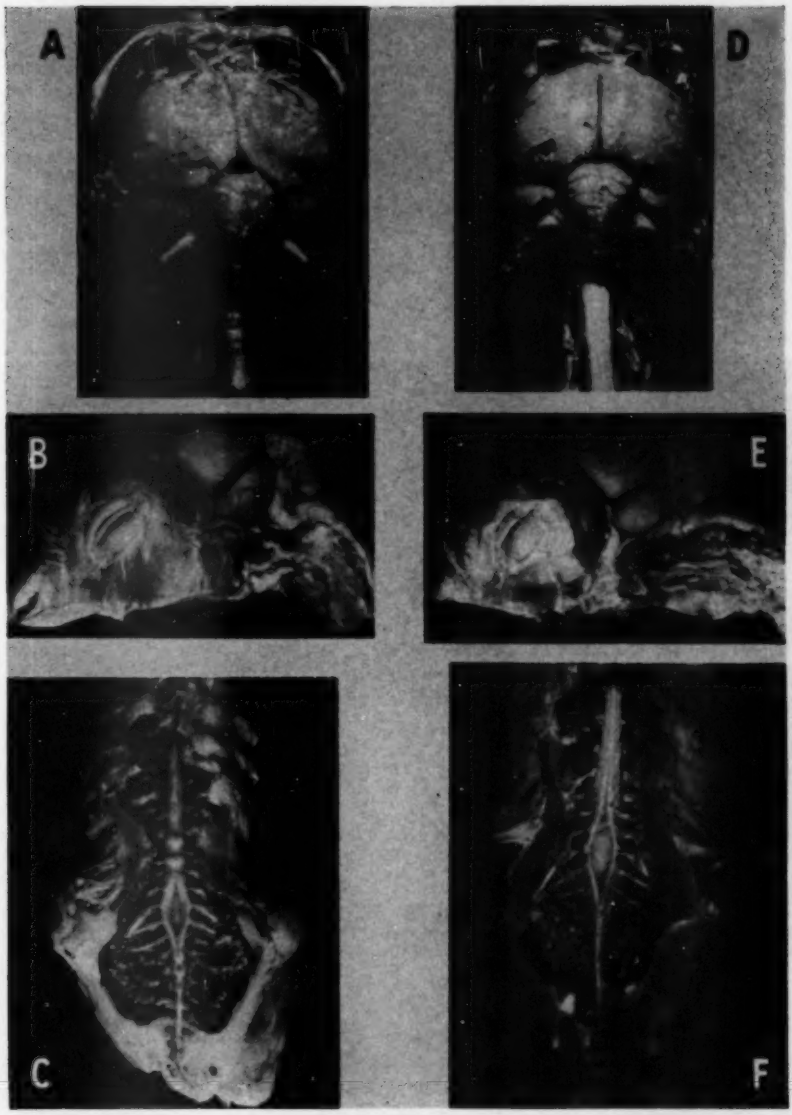

Fig. 2.—*A*, vitamin-A-deficient Chick 213. Note the shape of the cerebral hemispheres, the compression of the cerebellar folia, the distortion and elongation of the flocculi, and the molding of the medulla and the cervical spinal cord. See Figure 3*A* and *B* for brain herniating into the anterior confluence of vessels before the membranes were removed.

B, lateral view of the brain of Chick 214. Note the concave posterior surface of the cerebellum, the great prominence of the pontal flexure of the medulla, the molding of the medulla by the cerebellum, and the prominence of the tectum.

C, dissection of the lumbosacral spinal cord of vitamin-A-deficient Chick 214. The glycogen body has been removed. Note the transverse elevations of the spinal cord due to the molding of the cord into the roof of the synsacrum.

D and *E*, brain of a restricted-diet control chick. Compare this with the brain of the vitamin-A-deficient chick.

F, dissection of the lumbosacral spinal cord of a restricted-diet control chick. Compare the same regions in this and the vitamin-A-deficient chick. The glycogen body is in situ.

The restricted-diet control, Chick 474, is representative of four chicks whose food was reduced in amount on the 15th day of age. These four chicks were normal in all respects except for small size. Their central nervous systems were normally related to the spinal canals and cranial cavities, as seen on gross dissections (Fig. 2*D*, *E*, and *F*). The effect of the restricted diet upon endochondral bone growth will be described in our account of the microscopic studies which follows.

Histological studies were made of the soft tissues of only four chicks, Nos. 207, 255, and 473, listed in the table, and one other chick which was severely paralyzed when killed on the 27th day. All four of these chicks showed the normal epitheliums replaced by keratinizing epithelium in several locations, constantly in trachea and/or larynx, renal pelves and/or ureters, and esophageal glands. Other locations were conjunctiva, lingual glands, and bursa of Fabricius. In all instances, the replacement was focal, indicative of the early stages of the characteristic

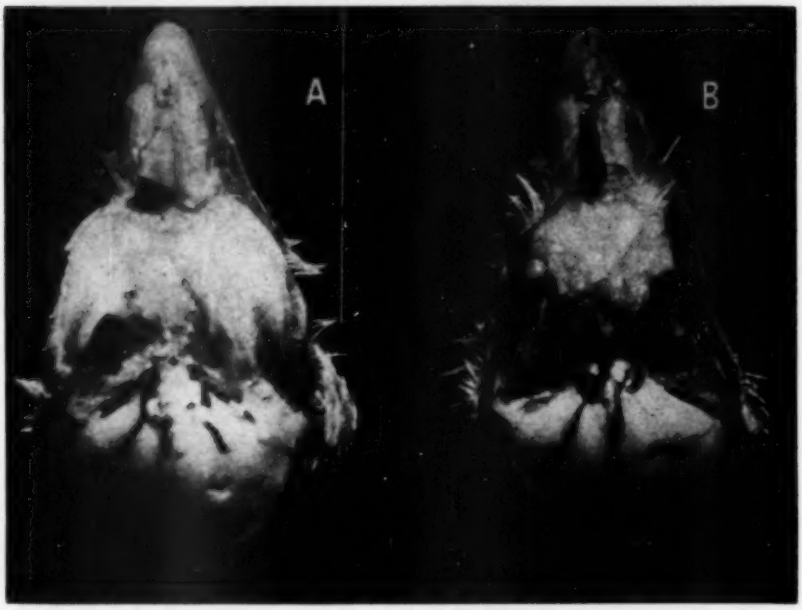

Fig. 3.—*A* is from Chick 214; *B*, from Chick 481. The photographs are enlarged to show how the brain herniates, via sites of arachnoidal villa, into the anterior confluence of vessels described in the text.

epithelial response to vitamin A deficiency. In the rat, also, the skeletal retardation can cause severe injury of the nervous system before the "keratinizing metaplasia" of epitheliums reaches an impressive degree.[1a]

GROSS PATHOLOGY

No attempt was made to compare the bones, divested of soft tissues, of the vitamin-A-deficient chicks with normal or restricted-diet controls. In the dissections of the spine and the skull, after fixation in 10% formalin (4% formaldehyde solution), whether decalcified (5% aqueous nitric acid) or not, a very evident decrease of density was noted in the vertebrae and the cancellous bones of the skulls of the

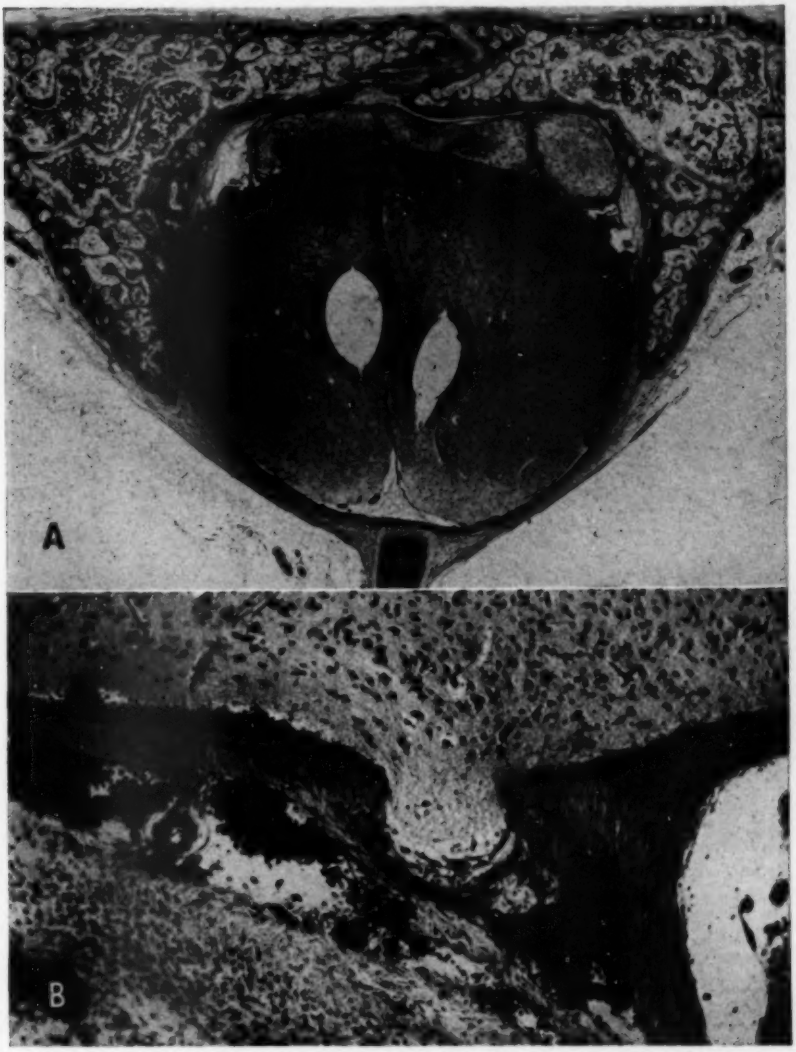

Fig. 4.—*A*, frontal plane; section through olfactory bulbs (lobes) showing herniations therefrom completely filling the anterior confluence of vessels; × 22. Note also the texture of the frontal bones typical of vitamin A deficiency. Compare with Figure 13*A* and *B*.

B, frontal plane section; level of the fenestrae ovales and the columellae; × 174. Note the small herniation of the ventral surface of the cerebrum in the temporosphenoidal sinus.

vitamin-A-deficient chicks compared with those of the normal and the diet-restricted controls.

Evidences of greatly disproportionate growth of the central nervous system and the axial skeleton were found in the cranial cavity and the spinal canal. The venous sinuses of the head and the tributary veins were always found to be greatly engorged as compared with those of normal chicks and of restricted-diet controls. This engorgement revealed, as far as we could ascertain, a hitherto-undescribed expansion of the longitudinal sinus at its anterior end where it receives veins from the surface of the brain, the nose, and the orbits. This confluence of vessels lies over the tips of the cerebral hemispheres and the olfactory bulbs or lobes. Arachnoidal villi enter into it precisely as the arachnoidal villi of man and the rat [18] enter the veins and

Fig. 5.—Vitamin-A-deficient Chick 240; longitudinal section through distal end of femur; × 21.5. Note failure of tunneling. Compare with a similar section, Figure 8, from a restricted-diet control chick.

sinuses of the dura. In the A-deficient chicks this confluence was found always to contain herniations of the cerebrum and olfactory bulbs and in some instances was almost completely filled by the herniations (Figs. 3*A* and *B* and 4*A*). Caps of arachnoidal cells were found upon the smallest herniations. No systematic search was made for brain tissue herniating into other venous sinuses; incidental to the study of frontal plane sections of the cranium and its contents, minute herniations of the ventral surface of the cerebrum were found in the temporosphenoidal sinus (Fig. 4*B*).

Comparison of the contents of the cranial cavities (after fixation *in situ*) of vitamin-A-deficient chicks, normal control chicks, and restricted-diet control chicks

revealed obvious compression effects in the vitamin-A-deficient chicks, in addition to the herniations seen in the anterior confluence of sinus and veins. Most conspicuous were the broadening of the hemispheres of the forebrain and the shortening of the longitudinal length of the brain as a whole. The angle formed by the pointed tips of the hemispheres was much wider, and the olfactory bulbs were distorted and flattened (Fig. 2*A*). Viewed laterally (Fig. 2*B*) the optic lobes (tecta) were more prominent and, when separated from the hemispheres, showed molding by the

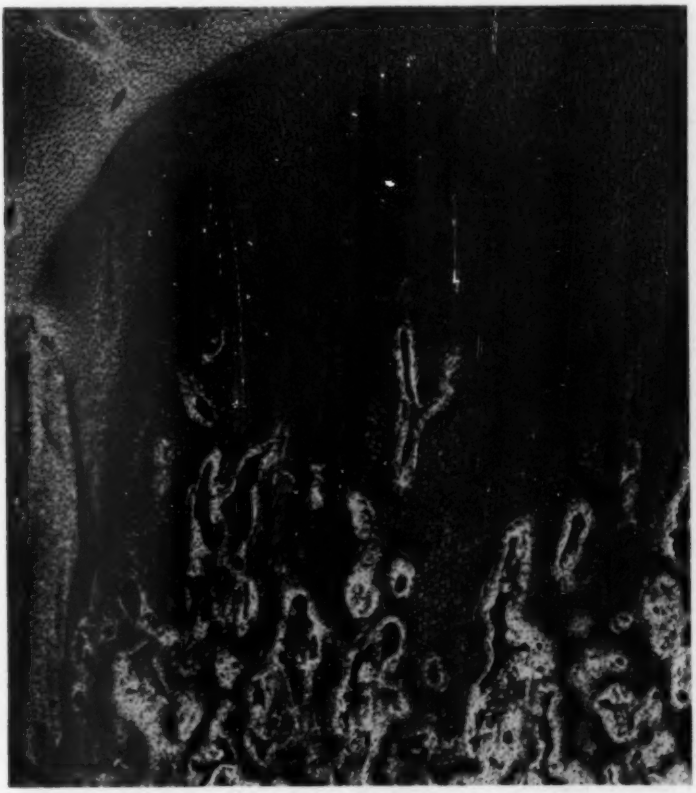

Fig. 6.—A higher-power view of a portion of the section shown in Figure 5; × 50. Note the deeper staining of the cartilage in the paths of advancing blood vessels and the great reduction of mature cells on the diaphyseal side. Compare with Figure 9, from a restricted-diet control chick, and with Figures 1 and 2 of the preceding paper,[2] showing corresponding sections made from a normal control chick.

posterior surfaces of the latter, indicated by a great increase in concavity of their anterior surfaces, which were bordered by sharp edges in contrast to the controls, where the anterior surfaces of the tecta were plane-like, with rounded borders. Alteration of the shape of the cerebellum viewed laterally was also very evident and included increase of its dorsoventral length, marked concavity of its posterior surface,

and molding into the medulla. Other evidences of compression of the cerebellum were slight extrusions into the fossae for the flocculi and elongation and flattening of the latter in the frontal plane. The fissures between the cerebellar folia were less evident than in the controls.

The medulla also (Fig. 2*A* and *B*), in all the A-deficient chicks, was compressed and molded into the foramen magnum. Its dorsal surface showed the imprint of the first cervical vertebra, and the upper cervical spinal cord also was similarly trans-

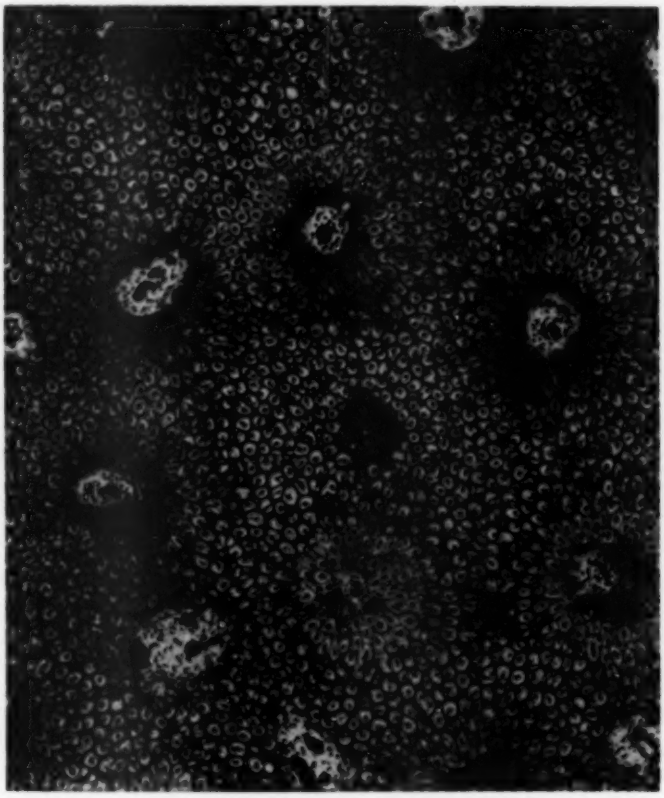

Fig. 7.—Cross section of the epiphyseal cartilage, distal end of femur, of vitamin-A-deficient Chick 385; × 120. Compare with the normal sections shown in Figure 4*A* and *B* of the preceding paper [3] and note wider spacing of the tunnels and decreased vascularity in the vitamin-A-deficient chick. The level of sectioning corresponds more nearly to that of *B* in Figure 4, from the normal.

versely molded by the yielding of the bone (shown in histological preparations) of the vertebrae to pressure. The ventral surface of the medulla showed small excrescences (herniations) at the origin of the vagus nerves. Viewed laterally, the A-deficient-chick medulla showed conspicuous enlargement of the pontal flexure (Fig. 2*B*).

Inspection of the ventral surface of the A-deficient brain showed equally prominent effects of compression and molding, such as the change in contour and dimensions of the forebrain, slight overriding of the medulla by the optic bulbs, and a displacement of the optic nerves and infundibulum in a ventral and caudad direction.

Evidences of compression of the spinal cord were found at all levels in the presence of some degree of transverse ribbing resulting from the yielding (resorption) of bone of vertebrae. In the lumbosacral spinal canal, where enlarged for housing of the glycogen body, a moderate degree of ribbing was present on the ventral surface of the spinal cord, permitted by the yielding (resorption) of bone of vertebral bodies, while a much more prominent ribbing both of spinal cord and glycogen body was present on the dorsal surface where these structures were molded

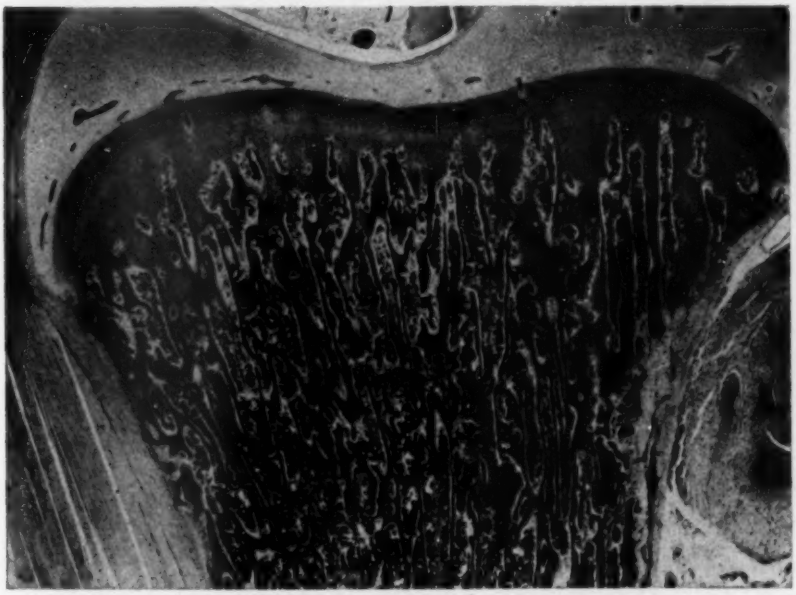

Fig. 8.—Longitudinal section of a restricted-diet control Chick, 474, whose weight graph is compared with those of vitamin-A-deficient chicks in Figure 1. Note the regularity of the tunnels, the clear demarcation of the epiphyseal cartilage zones, and the degree of ossification; × 22.5. Compare with Figure 5.

into the intervertebral transverse concavities normal to the roof of the synsacral enlargement of the spinal canal (Figs. 2*C* and 10*B*).

We could find no gross evidence of injury or distortion of the spinal nerve roots. It should be recalled that in the chick the spinal cord, unlike that of most mammals, extends the entire length of the spinal canal and that the nerve roots leave the spinal cord at right angles.[4]

4. Longitudinal serial sections were made of four lumbosacral spines of A-deficient chicks. Neither in these nor in transverse serial sections of lumbar vertebrae could we find evidence of mechanical pressure at foramens of exit. Degeneration of ganglion cells of root ganglions was found but could be explained as the result of central nervous system lesions caused by pressure.

MICROSCOPIC PATHOLOGY [5]

The Long Bones.—Comparison of normal and pathologic skeletal growth demands consideration of endochondral bone growth, appositional bone growth, compact bone formation, and remodeling sequences.

The most obvious effect of vitamin A deficiency upon bone growth in the chick [1a] (as in mammals), compared with the normal and restricted-diet controls, is upon

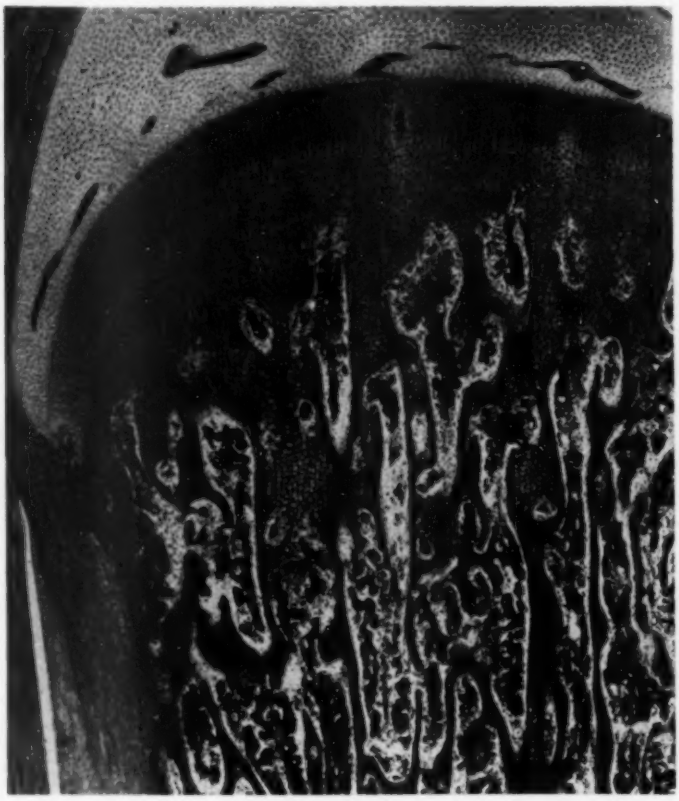

Fig. 9.—Higher-power view of a portion of the section shown in Figure 8; × 50. Compare with Figure 6. Note that in the restricted-diet control the degree of ossification is much greater than that shown in Figure 6, from the vitamin-A-deficient chick.

epiphyseal cartilage sequences and endochondral bone growth. The tunneling of the epiphyseal cartilage is irregular, often forked or branched, and less extensive (Fig. 5). The zone of proliferating cartilage cells is less clearly demarcated, and cells in mitosis are absent. The intercellular matrix gradually increases, and there

5. All histological descriptions were made from bones fixed in 10% formalin (4% formaldehyde solution), embedded in celloidin, and stained with hematoxylin and eosin.

is formed a broad zone of moderately enlarged cells in noncalcified matrix. Cells of mature size surrounded by calcified matrix constitute a narrow and irregular zone in the diaphyseal side where tunneling is present (Fig. 6). A longitudinal streaking of the epiphyseal cartilage, not apparent in normal or restricted-diet controls, is present in the A-deficient chicks and is produced by alternate zones of

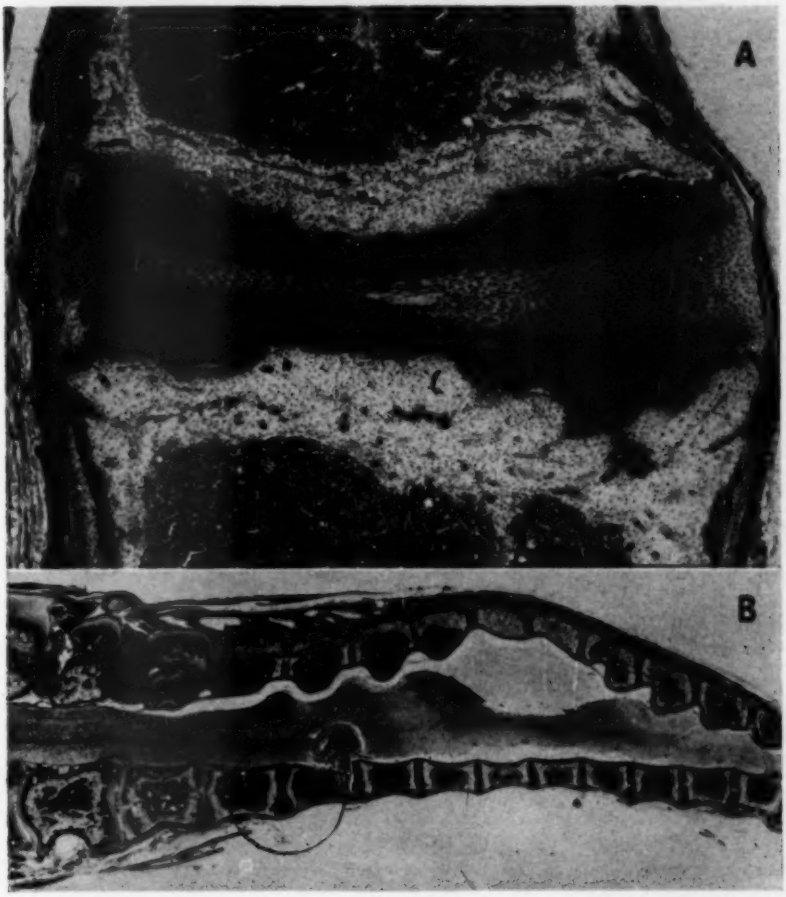

Fig. 10.—Sections from vitamin-A-deficient Chick 214: *A*, section of the cartilage between the first and second lumbosacral vertebrae; × 56.5. See ring in *B*. Note complete absence of endochondral bone growth activities, absence of trabecular bone, and thinness of cortical bone. Compare with Figure 11*A*.

B, longitudinal section of the synsacrum of vitamin-A-deficient Chick 214. Note the molding of the spinal cord and glycogen body described in the text. At the caudal end of the second lumbosacral vertebra the break with extrusion of nerve tissue is an artefact. Compare with Figure 11*B*.

maturing and nonmatured cells. Because these zones correspond in size with the dimensions of the tunnels in normal growth, it is our conviction that the retardation produced by the deficiency reveals evidence that the determining factor in the pattern of tunneling resides in the epiphyseal cartilage.

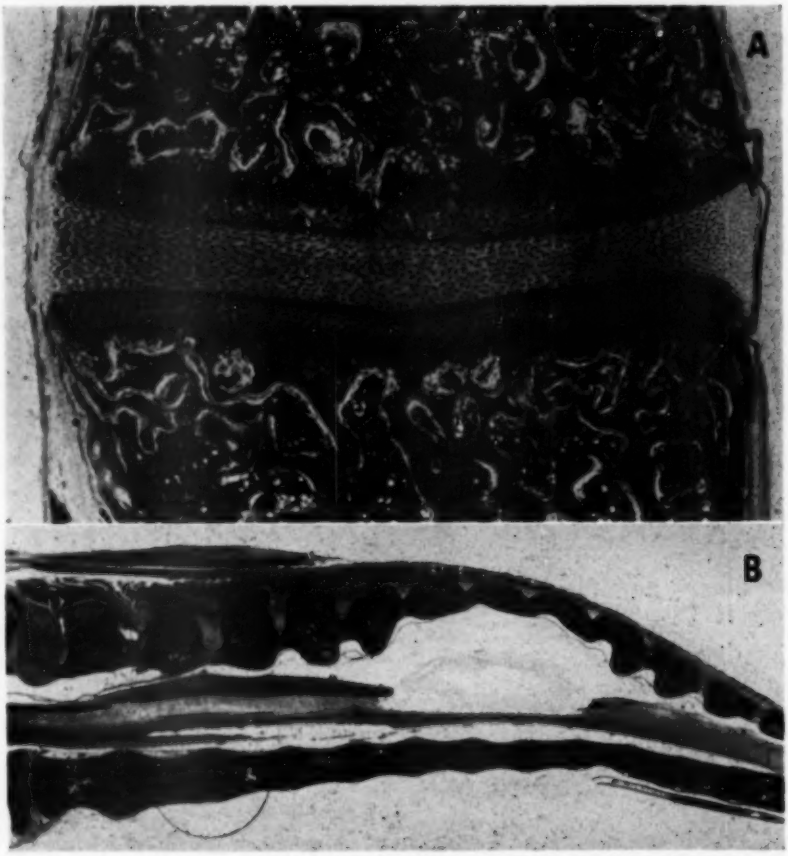

Fig. 11.—Sections from a normal control chick—Chick 431 of the preceding paper.[9] *A*, section of the cartilage between the first and second lumbosacral vertebrae; 56.5. See ring in *B*. Note the endochondral bone growth, abundant trabecular bone, and the cortical bone. Compare with Figure 10*A*.

B, longitudinal section of the synsacrum of Chick 431 (normal control); × 5. Compare with Figure 10*B*.

Detailed description of endochondral bone growth in the deficient chicks would be largely repetitious of that of normal chicks.[3] The changes are those of retarded multiplication, growth, and maturation of cartilage cells, delayed calcification of

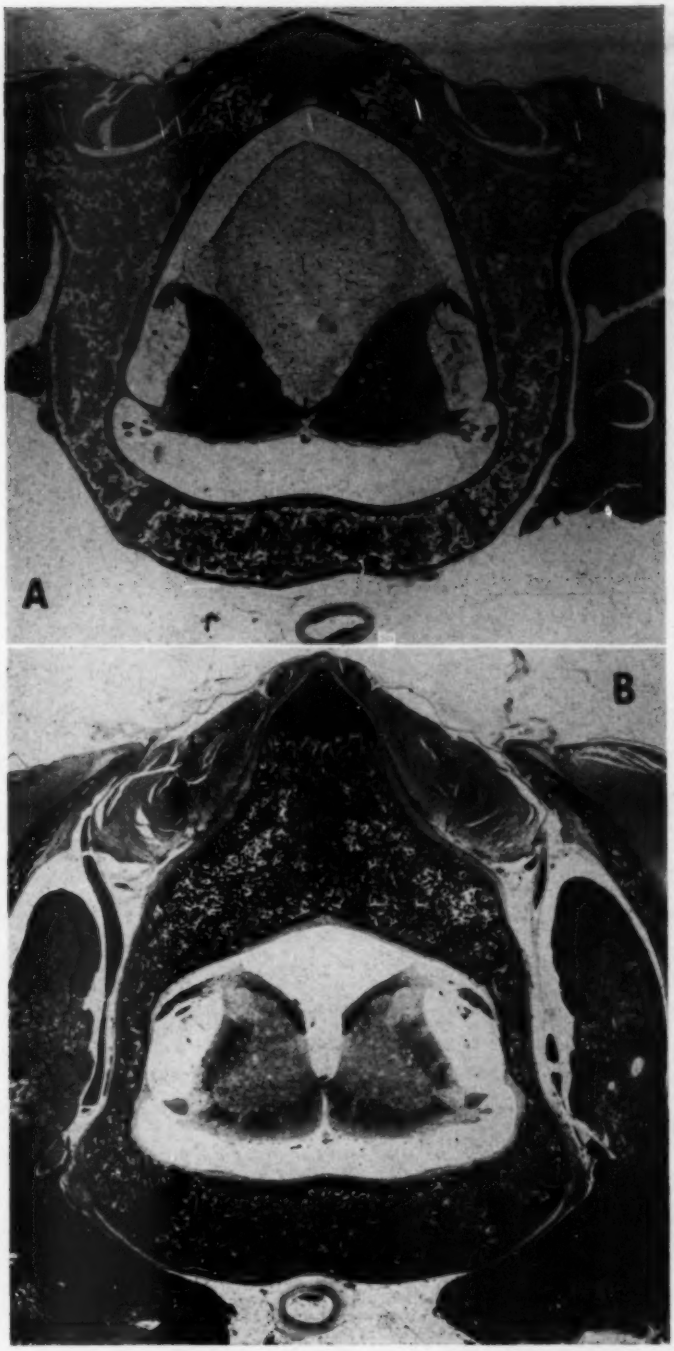

Fig. 12.—*A*, cross section of a lumbosacral vertebrae through the glycogen body of vitamin-A-deficient Chick 473; × 12.5. Note the texture of the bone.

B, cross section of seventh thoracic vertebra of a restricted-diet control chick; × 12.5. Compare *A* and *B*.

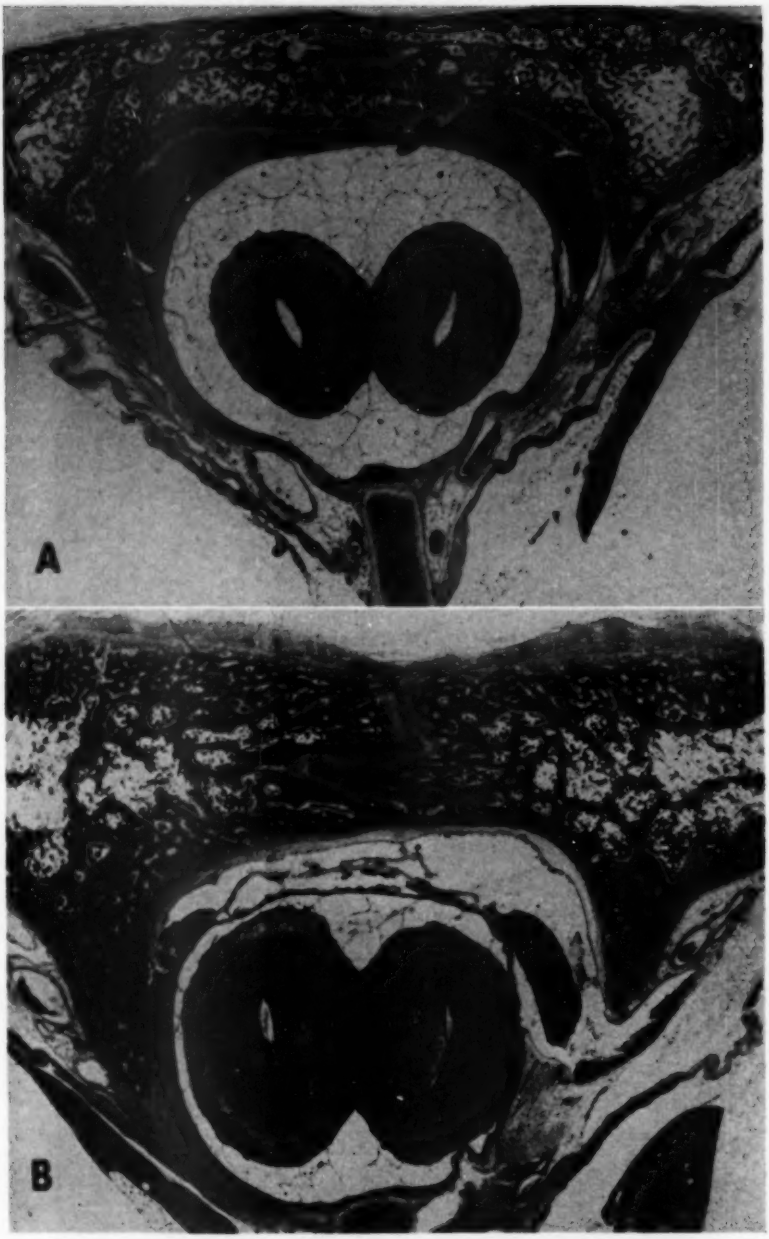

Fig. 13.—Frontal planes. Cross sections through the skull at the level of the olfactory bulbs (lobes) showing the anterior confluence of vessels. *A*, restricted-diet control, Chick 474; × 25. (See graph, Fig. 1.) Note the structure of the frontal bones and compare with the same regions in vitamin-A-deficient chick, Figure 4*A*, and the normal, Figure 13*B*.

B, similar section from a normal chick, 430, of the preceding paper.[3] Compare with Figures 4 and 13*A*.

cartilage matrix, and less evidence of chondrolysis in advance of and lateral to entering blood vessels (Figs. 6 and 7). These retarded sequences account for the less extensive tunneling of the epiphyseal cartilage.

Appositional bone growth is retarded earlier than in mammals, even in the chicks which were force-fed or killed before inanition became dominant. In all the deficient chicks, osteoblasts, periosteal and endosteal, were fewer and individually smaller than in restricted-diet controls. Much less cancellous bone was present in the metaphyseal region, and the adjacent cortical bone was thinner and less dense than in either normal chicks or restricted-diet controls (Figs. 8 and 9). In all the A-deficient bones, remodeling sequences had ceased, as shown by the great scarcity of osteoclasts.

Vertebrae and Bones of the Skull.—Longitudinal and cross sections of vertebrae showed, in all regions of endochondral ossification, retardation or complete inactivity of growth. Retardation and suppression of bone formation per se was more striking than in the long bones, so that the bodies of the vertebrae were very deficient in cancellous bone, and the cortical bone was thin and wholly devoid of compact bone (Figs. 10, 11, and 12*A* and *B*).

In frontal plane sections of the skull the findings were similar in regard to all aspects of bone growth (Figs. 4*A* and 13*A* and *B*). Sections with the brain *in situ* showed microscopic herniations of the brain into venous sinuses (Fig. 4*A* and *B*) in addition to the grossly visible herniations into the confluence of veins and sinus on the dorsal surface of the anterior tips of cerebral hemispheres. Tufts of arachnoidal cells could occasionally be found at the apices of herniations proving without question that the herniations were related to the arachnoidal villi present in sections of the same regions made from normal and restricted-diet control chicks.

COMMENT

In the account we have given, of the effects of vitamin A deficiency upon the growing skeleton of the chick, we have purposely omitted many histological minutiae seen in normal and deficient chicks not relevant to the comparison of essentials of skeletal growth. The retardation of all sequences of bone growth and especially of endochondral bone growth when examined with consideration of weight graphs and onset of paralyses (Fig. 1) satisfactorily proves that in chicks as in mammals there occurs a disproportionate growth of the central nervous system and its bony investment. Gross and microscopic examinations have yielded objective evidence of compression of the brain and spinal cord as a result of the disproportionate growth. In the cranial cavity the most apparent effects—the brain herniating into venous sinuses, the cerebellum distorted, and the medulla dislocated caudad into the foramen magnum—are strikingly similar to the consequences of the overcrowding of the human cranial cavity which results from the growth of intracranial tumors.[6]

The effect of the restricted diet upon skeletal growth can best be described as a balanced retardation of all growth sequences. The bones are smaller, but bone formation and bone resorption in remodeling maintain normal relations to each

6. Wolbach, S. B.: Multiple Hernias of the Cerebrum and Cerebellum Due to Intracranial Pressure, J. M. Res. **19**:153, 1908.

other. Epiphyseal cartilage cell growth and maturation are slowed down, but the zones of proliferation, growth, and maturation are well demarcated from one another and preserve essentially normal relationships. Appositional bone growth continues, conforming to endochondral bone growth, as does compact bone formation. The results are that the contours of all bones are normal and that the bones are sturdy structures in which there are normal relationships in configuration and in volume of metaphysis, cancellous bone, and cortical bone to one another (Figs. 8, 9, 12*B* and 13*A*).

Our findings are totally in variance with those of Adamstone,[7] who found no gross lesions of the central nervous system and stated that "while the skeletal structures of A-deficient chicks grow more slowly than normal, their brains are also much smaller. . . ."

Fletcher and Rigdon,[2a] Rigdon, Rude, and Bieri, [2c] and Rigdon [2d] in their studies of vitamin A deficiency in young ducks could find no evidence of effects caused by vitamin A deficiency upon skeletal growth adequate to explain the neurologic consequences. Fletcher and Rigdon [2a] and Rigdon [2d] did give some thought to skeletal growth and recorded the bizarre finding of cancellous bone with hematopoietic cells in the substance of the spinal cord in ducks maintained on a vitamin-A-deficient diet for 10 to 15 days. The photomicrographs submitted to document this phenomenon in both papers are more convincing of artefact than of osseous metaplasia in the central nervous system. They did consider mechanical compression of the spinal cord. Rigdon, Rude, and Bieri [2c] failed in their studies "to demonstrate in the skeleton of the duck either pathologic or roentgenographic changes that might be attributed to vitamin A deficiency."

It is impossible for us to appraise these three papers upon vitamin-A-deficient ducks because of the great differences in method of study employed by their authors and by us. It seems to us incredible that there should be greater differences between two avian species than between one avian species and the several mammalian species we have studied.[8] As brought out by Lubosch,[9] the patterns of bone growth of all birds are similar and have greater resemblance to those of cold-blood vertebrates than to those of mammals.

SUMMARY AND CONCLUSIONS

The results of vitamin A deficiency upon skeletal growth in young chicks are, except for differences in the patterns of endochondral bone growth, similar to those in young mammals.

All sequences concerned in skeletal growth are retarded before growth as a whole (estimated by weight increase) is materially affected and before there is extensive replacement of glandular epitheliums by stratified keratinizing epithelium.

Linear growth of long bones and the vertebral column and the growth of the cranium become retarded and finally completely suppressed by failure of endochondral bone growth.

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7. Adamstone, F. B.: Histologic Comparison of the Brains of Vitamin A Deficient and Vitamin E Deficient Chicks, Arch. Path. **43**:301, 1947.

8. Experiments upon ducklings fed vitamin-A-deficient diets have been begun by us and will be reported later.

9. Lubosch, W.: Die Bildung des Knochenmark beim Hünchen und bei Säugetieren und das Wesen des endochondralen, Ossifikation in historischen Betrachtung, Morphol. Jahrb. **53**:49, 1923-1924.

All epiphyseal cartilage cell sequences are retarded, namely, multiplication, growth, and maturation. Concurrently and in consequence there results retardation of tunneling and of ossification. In vertebrae and skull, complete suppression of endochondral growth may occur.

In contrast to mammalian bone growth, appositional bone formation ceases early, so that localized appositional bone growth concerned in the formation of some normal bony contours is not found disproportionate to endochondral bone growth.

The effect upon epiphyseal cartilage is distinctive and specific and is a primary result of vitamin A deficiency in the sense that it is not secondary to inanition.

The neurologic disturbances of vitamin A deficiency in the chick are the result wholly of compression of the central nervous system produced by retardation of growth of vertebrae and bones of the cranium.

The histological preparations were made by Mr. John J. Burke, departmental technician. The photomicrographs were made by Mr. John Carabitses, of the Department of Pathology, The Children's Hospital.

HYPERVITAMINOSIS A AND THE SKELETON OF GROWING CHICKS

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AND
D. MARK HEGSTED, Ph.D.
BOSTON

THE PURPOSE of this report is to record the effects of hypervitaminosis A upon the growth of the bones in young chicks.

The effects of the administration of excessive amounts of vitamin A upon the growth of bone have been described for several mammalian species: rat, mouse, guinea pig [1] and dog.[2] These effects [1a] are the acceleration of all processes of bone growth—epiphyseal cartilage sequences concerned in endochondral bone growth, remodeling sequences involving resorption of bone and appositional bone formation, osteogenesis per se, and compact-bone formation. The rate and the degree of the acceleration were found to bear no relation to linear growth of long bones but were directly related to the amounts of vitamin A administered.[1a,b]

Although only a few chicks (four) were studied histologically and only a few bones from each—the bones of the knee joints (femur, fibula and tibiotarsus), the hock joints (tibiotarsus and tarsometatarsus), vertebrae, and the skull, including those of the calvarium, the sphenoid, occipital and temporal bones—the findings were so consistent and so decisive that further processing of additional chicks seemed unnecessary.

Recently Rigdon, Rude, and Bieri [3] failed to find any effect of hypervitaminosis A upon growth of bones in young ducks.

EXPERIMENTAL STUDY

The diet, plus 1% cod liver oil, given from the first or second day after hatching, was that described in our account of endochondral bone growth in the chick.[4]

This study was aided by research grants from The Nutrition Foundation, the Williams-Waterman Fund for the Combat of Dietary Disease, and Swift and Company, Chicago.

From the Division of Nutritional Research, Division of Laboratories and Research of The Children's Hospital; the Department of Nutrition, Harvard School of Public Health, and the Department of Biological Chemistry, Harvard Medical School, Boston.

1. (*a*) Wolbach, S. B.: Vitamin A Deficiency and Excess in Relation to Skeletal Growth, Proc. Inst. Med. Chicago **16**:116, 1946; J. Bone & Joint Surg. **29**:171, 1947. (*b*) Von Metre, T. E., Jr.: The Influence of Hypervitaminosis A on Bone Growth, Bull. Johns Hopkins Hosp. **81**:305, 1947. (*c*) Wolbach, S. B., and Maddock, C. L.: Hypervitaminosis A: An Adjunct to Present Methods of Vitamin A Identification, Proc. Soc. Exper. Biol. & Med. **77**:825, 1951.

2. Maddock, C. L.; Wolbach, S. B., and Maddock, S.: Hypervitaminosis A in the Dog, J. Nutrition **39**:117, 1949.

3. Rigdon, R. H.; Rude, J. C., and Bieri, J. G.: Effect of Hypervitaminosis A and Hypovitaminosis A on the Skeleton of a Duck, A. M. A. Arch. Path. **52**:299, 1951.

4. Wolbach, S. B., and Hegsted, D. M.: Endochondral Bone Growth in the Chick, A. M. A. Arch. Path., this issue, p. 1.

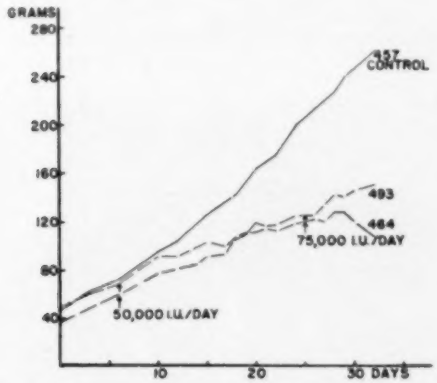

Fig. 1.—Graph to illustrate the growth of hypervitaminosis-A chicks, No. 464 and No. 493, in comparison with a normal control chick, 457.

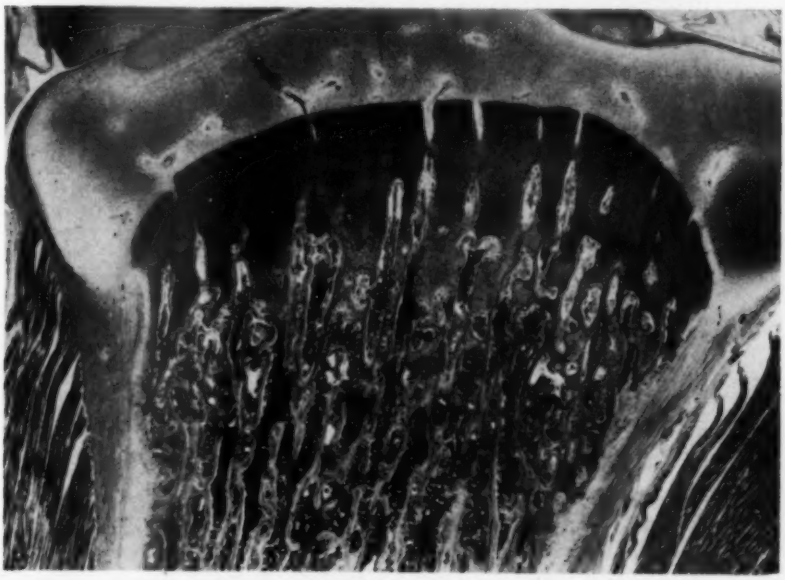

Fig. 2.—Distal end of femur of hypervitaminosis-A Chick 493, killed at 32 days of age; × 21.6. (See graph, Fig. 1.) Compare with the normal in Figure 1 of a preceding paper on endochondral bone growth in the chick.[4]

Beginning on the sixth or seventh day after hatching, when the weights of the chicks were 55 to 75 gm., 50,000 I. U. vitamin A were given daily by medicine dropper so that the dosages ranged from approximately 660 I. U./gm. to 900 I. U./gm. daily. After 20 days the dosage of two chicks, each weighing 125 gm., was increased to 75,000 I. U. daily (600 I. U./gm.). One chick (initial weight, 50 gm.) was killed in poor condition after 11 days on the 50,000 I. U. regimen.

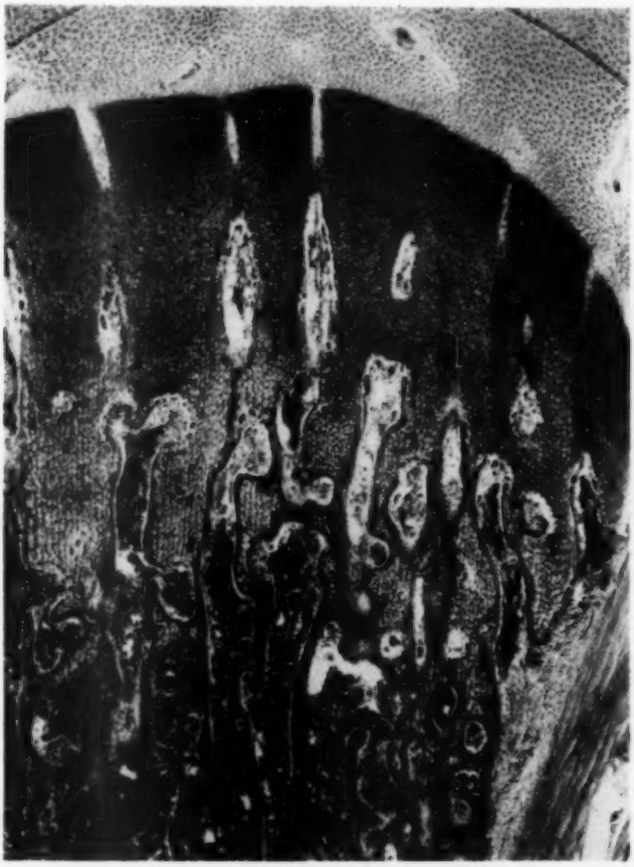

Fig. 3.—Higher-power view of a portion of Figure 2; × 43.75. Compare with the corresponding photomicrograph of the normal, illustrated in Figure 2 of a preceding paper.[4] Note the diminution of the width of all layers of the epiphyseal cartilage, the more extensive tunneling, the greater consumption of the tunnel walls, and the extent and volume of ossification.

The clinical signs of the hypervitaminosis A were retardation of weight gain with final weight loss (Fig. 1). Swelling and crusting of the eyelids and inflammatory lesions of the nares, the mouth, and adjacent skin, and of the skin of the feet were other signs.

The gross postmortem findings were those of emaciation. No fractures were demonstrated. We neglected to investigate the deformed feet of two chicks, which could have been the result of fracture or of luxation of phalanges.

MICROSCOPIC PATHOLOGY [5]

The Long Bones.—Epiphyseal Cartilage: There is a narrowing of all zones of the epiphyseal cartilages. The zone of proliferation is less clearly defined than in the normal, because there is earlier diaphysealward increase in matrix than in normal control chicks. Mitotic figures are about as numerous as in normal controls, so that the narrowing of this zone is evidently the result of more rapid growth of

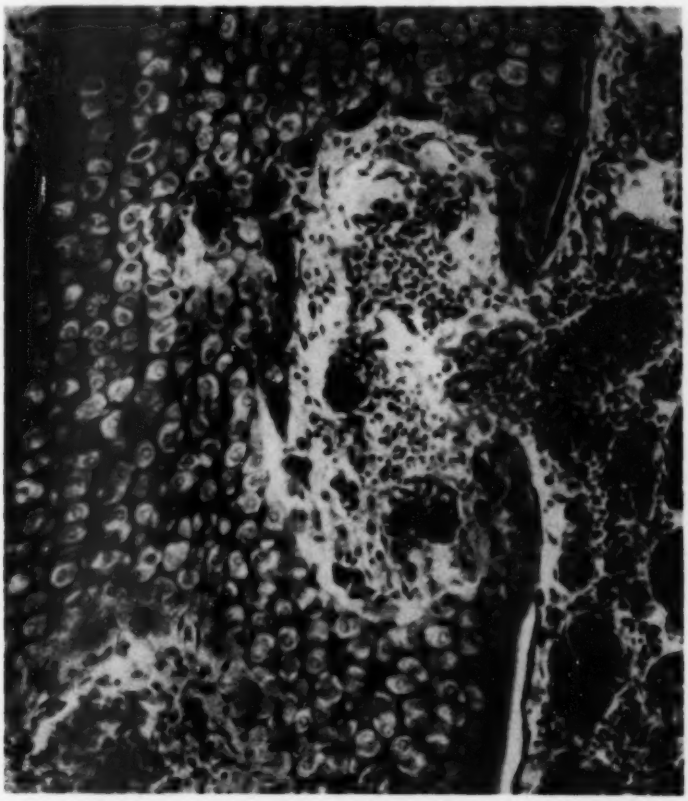

Fig. 4.—Higher-power detail of Figure 2 to illustrate the lateral excavation of the wall of a tunnel after deposition of bone; × 300. Note the multinucleated giant cells and the clearing of surrounding cartilage cells, apparently by chondrolysis. On the extreme left the ossified border of the adjacent tunnel is shown. This region is shown in Figure 2 just to the left of the midline. Compare with Figure 7 of the paper on endochondral bone growth in the chick.[4]

the cells. The zone of growth is narrowed because it rapidly merges into the zone of maturation. The tunneling of the cartilage, which is evenly placed, extends the full width of the zone of matured cells and thus approaches much closer to the distal

5. All descriptions were made from formalin-fixed bones, embedded in celloidin, and stained with hematoxylin and eosin.

margin of the epiphyseal cartilage than in the normal. Lateral excavations of the tunnels are present throughout the zones of matured cells (Figs. 2, 3, and 4).

Cross sections of the epiphyseal cartilage made through the advancing ends of the tunnels show an increase in the size of the blood vessels, a great increase in the diameters of the tunnels, and the early establishment of communications between tunnels as compared with corresponding sections of normal control chicks. Other features seen in cross section at this level are somewhat wider zones of chondrolysis and widespread maturation of cartilage cells as shown by disappearance of cytoplasm, shrunken, deeply stained nuclei, and deeply stained capsules (Fig. 5*A* and *B*). Osteoid deposits on the walls of the tunnels at corresponding levels are much more abundant than in normal controls (Figs. 3 and 4). Multinucleated giant cells—chondroclasts and osteoclasts—engaged in their respective activities are much more numerous than in the normal controls. Complete consumption of the cartilage by the concurrent processes of resorption and osteogenesis, and the formation of cancellous bone, rapidly succeed the zone of maturation. The result is that cancellous bone and marrow cells are found much closer to the epiphyseal end than in normal specimens.[6]

The rapid appearance of marrow cells of all types in advancing ends of tunnels may be regarded as one of the accelerations of hypervitaminosis A.

Remodeling Sequences: That remodeling sequences are more active than in normal bones is evidenced by an increase of osteoclasts in relation to both trabecular bone and cortical bone adjacent to the epiphyseal ends.

Periosteal Bone Formation: The deep layer of the periosteum is less cellular than that of normal controls. More and denser cortical bone has been deposited than in the normal chicks at corresponding age periods, and the appearances suggest those of bone approaching completion of growth.

Cortical Bone: In chicks of the same early age, the cortical bone of the hypervitaminosis-A chick is exceedingly dense (Fig. 6*A* and *B*). In all regions we have studied, compact bone rapidly replaces cancellous bone. Haversian systems mature rapidly, and in the short period of our experiments the cortical bone acquired the density of adult avian bone.

The Axial Skeleton.—In vertebrae and the skull the outstanding feature is the density of the bone, which can be attributed to the compact bone replacing cancellous bone. This replacement we have found in the calvarium and in bones of the base of the skull, the bony capsule of the internal ear, the ossicle (columella), and the bodies and processes of the vertebrae (Fig. 7*A* and *B*).

Endochondral ossification in the bones of the base of the skull and in vertebrae is characterized by the same features described in our account of the epiphyseal cartilage of long bones, though less evident because of the slower rate of growth normal to skull and vertebrae.

6. The epiphyseal cartilage of undernourished chicks used for controls to vitamin A deficiency studies at low magnifications bears a superficial resemblance to that of hypervitaminosis-A chicks because of the narrowing of the epiphyseal cartilage as a whole. In the undernourished chicks, the demarcation between proliferative and growth zones is sharply defined. The cells of the growth zone show little matrix, while the zone of matured cells is largely consumed by the sequences of osteogenesis. Mitotic figures could not be found in the zone of proliferation. (See Figs. 8 and 9 of the preceding paper, A. M. A. Arch. Path., this issue, p. 13).

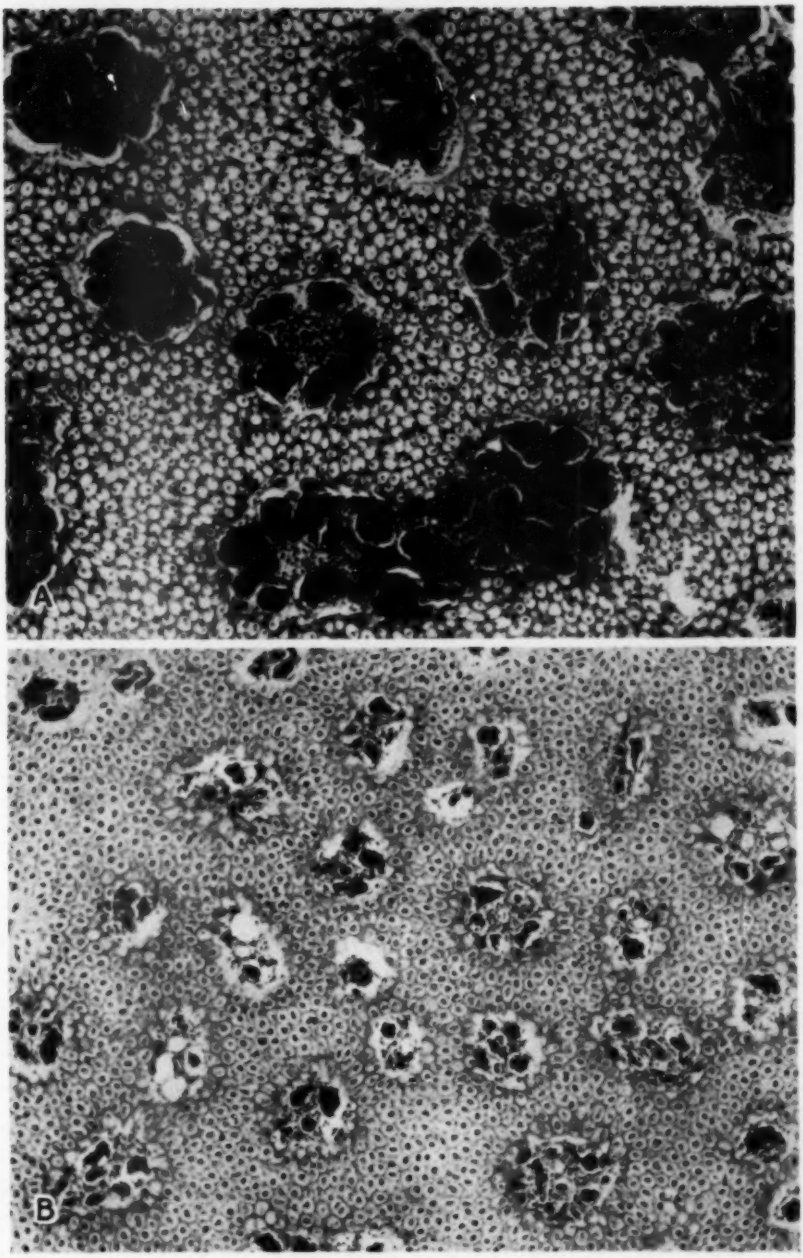

Fig. 5.—*A*, cross section of advanced cartilage tunnels in hypervitaminosis A; distal femoral epiphysis of a 26-day old chick; × 120. Compare with the normal shown in *B*.

B, corresponding level of tunneling in a normal chick 22 days old; distal femoral epiphysis of Chick 414.[4] × 120.

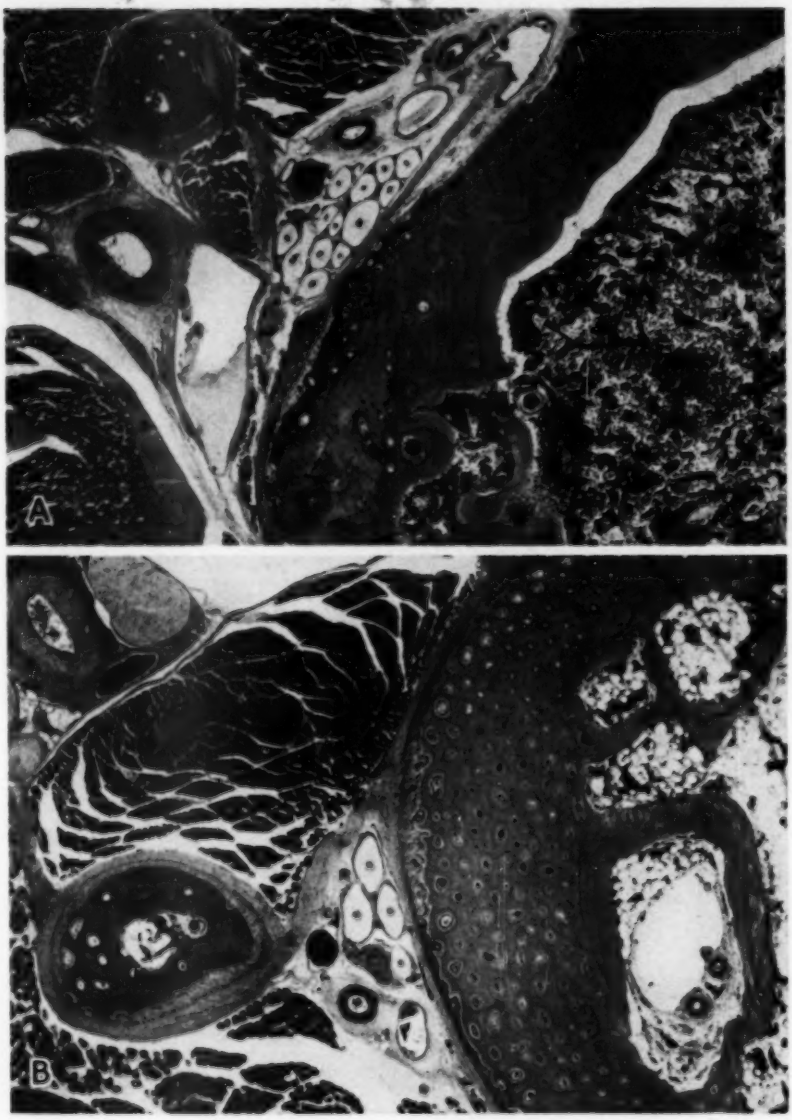

Fig. 6.—*A*, cross section of the tibiotarsus and fibula of hypervitaminosis-A Chick 464. (See graph, Fig. 1.) Note the density of the cortical bone. Compare with *B*, from a normal age-control chick which weighed 212.5 gm.

B, cross section of the tibiotarsus and fibula of a normal age-control chick made at a level corresponding to that of *A*. Note the texture of the cortical bone and the continued activity of periosteal bone formation.

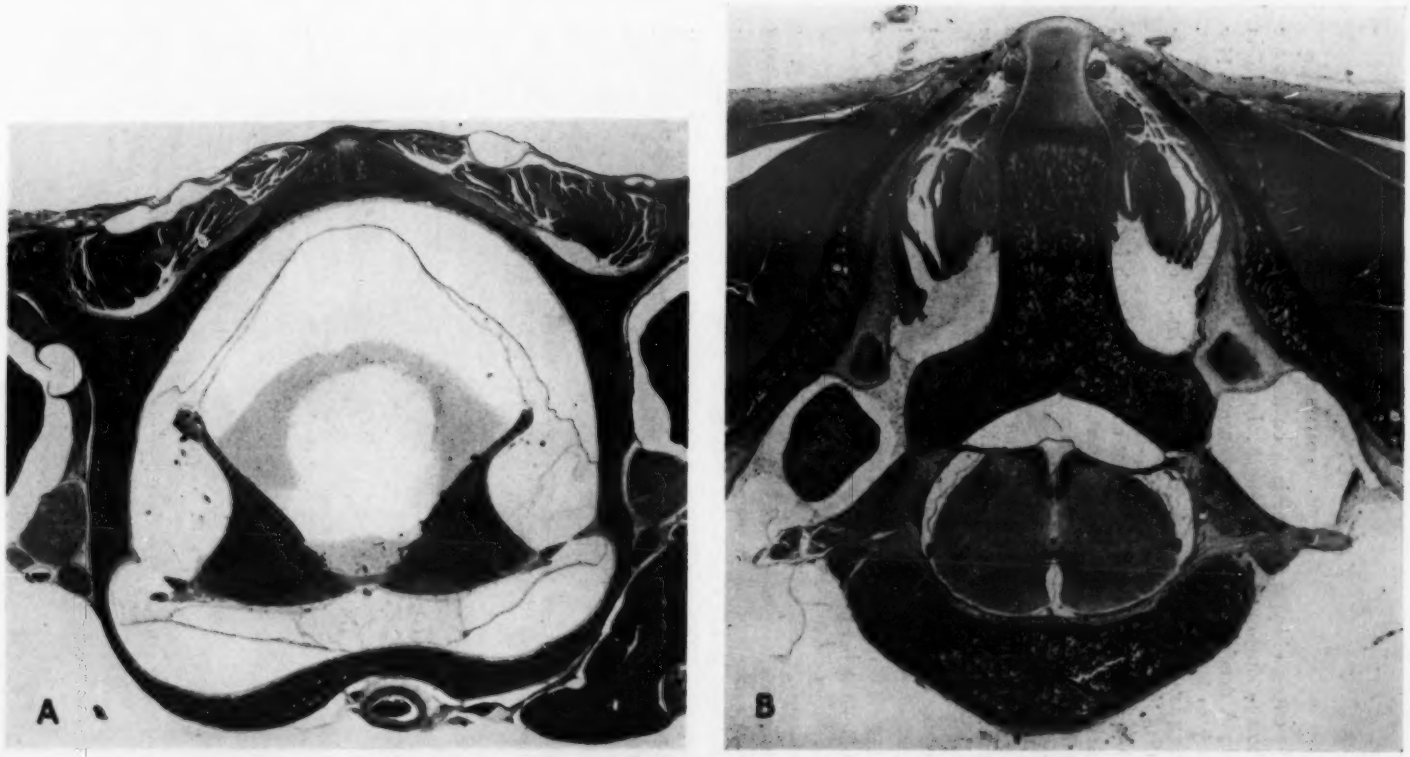

Fig. 7.—*A*, cross section of the spinal canal—synsacrum—through the sixth lumbosacral vertebra; × 12.5. (See Fig. 11*B* of the preceding paper, "Vitamin A Deficiency in the Chick" [A. M. A. Arch. Path., this issue, p. 13]). Note the density of the bone. Compare with *B*.

B, cross section of the seventh thoracic vertebra of normal Chick 430,[3] 22 days old; × 12.5. Note texture of the bone.

COMMENT

The effects of hypervitaminosis A on the skeletal growth of young chicks are like those produced in young mammals. In general, all those sequences retarded in vitamin A deficiency are accelerated, namely, epiphyseal cartilage sequences preliminary to bone replacement, endochondral bone growth, remodeling processes, and compact-bone formation. Acceleration of the last is outstandingly more prominent than in rats and guinea pigs.

The pattern of bone growth is not changed, but each feature of the composite is caused to proceed at a rate much more rapid than is normal. No other vitamin given in excess of physiologic needs has a similar effect upon the growth sequences of any structure.

Recently we have shown in rats [7] that the skeletal responses to hypervitaminosis A are not mediated through the anterior pituitary and probably not through the adrenal cortex.

Because the hypervitaminosis-A bone effects, like the patterned growth of fetal bone, are unrelated to concurrent functioning of the skeleton and because postnatal bone growth is plainly a continuation of fetal growth, we cannot avoid the inference that vitamin A has properties like those of inductor or evocator agents.

CONCLUSIONS

The skeletal responses to hypervitaminosis A in young chicks are precisely of the same nature as those in mammals, though differences in the pattern of endochondral bone growth must be considered.

As in mammals, hypervitaminosis A in growing chicks accelerates all histological sequences concerned in bone growth in conformity with the normal growth patterns.

Mr. John J. Burke, departmental technician, made the histological preparations. Mr. John Carabitses, of the Department of Pathology, The Children's Hospital, made the photomicrographs.

7. Wolbach, S. B., and Maddock, C. L.: Vitamin-A Acceleration of Bone Growth Sequences in Hypophysectomized Rats, A. M. A. Arch. Path. **53**:273, 1952.

PSEUDOMEMBRANOUS COLITIS FOLLOWING AUREOMYCIN AND CHLORAMPHENICOL

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DIARRHEA is a frequent effect of aureomycin and chloramphenicol.[1] Associated with such diarrhea in some cases which we have encountered was pseudomembranous colitis. That there was a relationship between the antibiotic therapy and the pseudomembranous colitis was suggested by a sudden increase of the latter as an incidental finding at autopsy. Subsequent clinical attention to the symptoms and signs of colitis, several x-ray observations,[2] and the identical colonic changes demonstrated in a surgical specimen of a patient who survived, strengthened the suspicion originally aroused in the autopsy room.

The changes found in the colon are not specific. They resemble those found in bacillary dysentery,[3] in postoperative states,[4] in paratyphoid colitis,[5] in septicemias (streptococcus, proteus, pyocyaneus), in toxic states (uremia, mercury, arsenic, bismuth),[5] and perhaps in "pseudodysentery" of starvation victims.[6] There are a few

From the Department of Pathology, Beth Israel Hospital, and the Department of Pathology, Harvard Medical School, Boston.

1. (*a*) Harris, H. J.: Aureomycin and Chloramphenicol in Brucellosis, with Special Reference to Side Effects, J. A. M. A. **142**:161, 1950. (*b*) Altemeier, W. A., and Culbertson, W. R.: Chloramphenicol (Chloromycetin) and Aureomycin in Surgical Infections, ibid. **145**:449, 1951. (*c*) Harvey, J. C.; Mirick, G. S., and Schaub, I. G.: Clinical Experience with Aureomycin, J. Clin. Invest. **28**:987, 1949. (*d*) Brainerd, H.; Lennette, E. H.; Meiklejohn, G.; Bruyn, H. B., Jr., and Clark, W. H.: The Clinical Evaluation of Aureomycin, ibid. **28**:992, 1949. (*e*) Finland, M.; Collins, H. S., and Paine, T. F., Jr.: Aureomycin, a New Antibiotic: Results of Laboratory Studies and of Clinical Use in 100 Cases of Bacterial Infections, J. A. M. A. **138**:946, 1948.

2. Fleischner, F. G., and Sedlezky, I.: To be published.

3. Fischer, W.: Ruhr und asiatische Cholera, in Handbuch der speziellen pathologischen Anatomie und Histologie, edited by F. Henke and O. Lubarsch, Berlin, Springer-Verlag, Vol. IV/3, pp. 417-468, 1929.

4. (*a*) Dixon, C. F., and Weisman, R. E.: Acute Pseudomembranous Enteritis and Enterocolitis: A Complication Following Intestinal Surgery, S. Clin. North America **28**:999, 1948. (*b*) Penner, A., and Bernheim, A. I.: Acute Postoperative Enterocolitis: A Study on the Pathologic Nature of Shock, Arch. Path. **27**:966, 1939.

5. Siegmund, H.: Einfache Entzündungen des Darmrohres, in Handbuch der speziellen pathologischen Anatomie und Histologie, edited by F. Henke and O. Lubarsch, Berlin, Springer-Verlag, Vol. IV/3, pp. 261-370, 1929.

6. Maladie de famine: Recherches cliniques sur la famine executées dans le Ghetto de Varsovie en 1942, edited by E. Apfelbaum, Warsaw, American Joint Distribution Committee, 1946.

gross and histologic similarities with colonic changes observed after ionizing radiation.[7] The reasons for attributing to aureomycin and chloramphenicol pathogenetic power with respect to the pseudomembranous colitis encountered are largely circumstantial, and are as follows:

1. The colitis did not appear in epidemic form.
   The disease occurred under hospital supervision.
   There was no antecedent history, prior to hospitalization, of diarrhea, tenesmus, or bloody or mucoid discharge.
2. No pathogenic micro-organisms were isolated.
   Amebae were not demonstrated in the sections.
   Fungi were generally absent in Gram-stained sections.
3. No previously known nonbacterial agents, such as heavy metals or uremia, could be incriminated.
4. One could not accuse any of the various other drugs which these patients received since none has been known, in the past, to be capable of inducing colitis.[8]
5. Every patient in this series had received aureomycin, chloramphenicol, or both; diarrhea did not occur until after use of drugs had been started.
6. All the cases presented morphologic changes which constituted a coherent sequence.

SELECTION OF CASES

Of 213 adults on whom autopsies were done from January, 1949, to November, 1950, 49 had received aureomycin or chloramphenicol or both. Pseudomembranous colitis was present in five; four of the five had received aureomycin, and one, chloramphenicol (Table).

Common to these 5 autopsy subjects was, first, that they had received either in excess of 4.0 gm. of aureomycin hydrochloride or chloramphenicol by mouth or in excess of 2.0 gm. of aureomycin hydrochloride by vein, and, second, that they had survived more than four days after the first administered dose. Accepting these figures as "significant" with respect to colitis developing after antibiotic treatment, there were, nevertheless, 26 patients similarly treated in whom pseudomembranous colitis did not develop. Eighteen patients less vigorously treated also were without this pathologic condition (Table).

The sex factor in the relationship of pseudomembranous colitis and antibiotic therapy is distinct (Table). Although the total number of males receiving "significant" doses was more than one and one-half times as large as that of females, pseudomembranous colitis was rare in the former (1 of 19) and frequent in the latter (4 of 12).

Several patients who had received "significant" doses of antibiotics had, on microscopic examination, hyperproduction of tenacious mucus in the colon (colica mucinosa [5]) and even in the small intestine; yet, the mucous membranes of the

7. (*a*) Liebow, A. A.; Warren, S., and DeCoursey, E.: Pathology of Atomic Bomb Casualties, Am. J. Path. **25**:853, 1949. (*b*) Friedman, N. B.: Effects of Radiation on the Gastrointestinal Tract, Including the Salivary Glands, the Liver and the Pancreas, in Warren, S.: Effects of Radiation on Normal Tissues, Arch. Path. **34**:749, 1942.

8. A possible exception is penicillin, which was administered to most of the patients herein reported. W. E. Herrell (Penicillin and Other Antibiotic Agents, Philadelphia, W. B. Saunders Company, 1945) mentioned that, particularly in women, penicillin alone can lead to colicky pains even to the point of imitating a surgical abdomen. However, we do not recall ever having encountered colitis that could be attributed to penicillin.

intestines looked quite normal grossly. Some of these patients had shown transient diarrhea following "significant" doses of antibiotics. Unfortunately, only rarely were tissues preserved from intestines which looked grossly normal. It appears possible that this excess mucous secretion would have been encountered more frequently had such tissues been taken with greater regularity and that this colica mucinosa represents the mildest response made to injury from antibiotic therapy.

PATHOLOGIC FINDINGS

Grossly, the disease affects the colon only. It presents elevated plaques of yellow to gray color, which are soft and moist in the early cases (Fig. 1), drier and firmer in the later ones (Fig. 7). The mucosa may be involved diffusely (*a* in Fig. 9*A*) or in plaques which range in size from 0.1 to 0.6 cm. and which may be confluent (Fig. 1) or discrete (Figs. 8 and 9*B*). In some cases the plaques are evenly distributed; in others they concentrate in the proximal portions of the colon. Some can be scraped off easily; others, with difficulty.

Sex Distribution of Forty-Nine Patients Receiving Aureomycin and/or Chloramphenicol According to Dose and Absence or Presence of Pseudomembranous Colitis

	Patients Receiving "Significant Doses" * of Antibiotic				Patients Receiving Less Than "Significant Doses" of Antibiotic	
	Males		Females			
	Pseudomembranous Colitis Absent	Pseudomembranous Colitis Present	Pseudomembranous Colitis Absent	Pseudomembranous Colitis Present	Males, Pseudomembranous Colitis Absent	Females, Pseudomembranous Colitis Absent
Aureomycin	13	1	7	3	7	10
Aureomycin plus chloramphenicol	3	0	0	0	0	0
Chloramphenicol	2	0	1	1	1	0
Total	18	1	8	4	8	10

* Under "Significant Dose" is meant survival of at least four days following the first dose together with (a) at least 4.0 gm. of aureomycin hydrochloride or chloramphenicol perorally or (b) at least 2.0 gm. of aureomycin hydrochloride intravenously.

Microscopically, there is a distinct sequential pattern of evolution, although there is much overlapping of the various histological phases in a given case.

Total goblet cell transformation and mucous hyperproduction of the gland and surface epithelium are seen early. The mucous product appears tenacious and is retained between exaggerated mucosal folds (Fig. 6*B*). This picture matches the colica mucinosa of patients mentioned previously who had received "significant" doses of antibiotics and in whom diarrhea developed but in whom the changes described hereafter did not evolve.

Mucous exhaustion of the epithelium is more common. Mucous masses overlie a mucosa totally devoid of goblet cells and lined, instead, by a compact, well-polarized epithelium, which appears original rather than regenerated (Figs. 2*A* and 10*A*). This interpretation of mucous exhaustion is based on the life cycle of the goblet cell as depicted by Maximow and Bloom[9]: "A goblet cell seems to pass many times through the successive phases of secretory activity until it finally perishes and is shed. Mitoses have been observed occasionally in them. As a rule, however,

9. Maximow, A. A., and Bloom, W.: A Textbook of Histology, Ed. 4, Philadelphia, W. B. Saunders Company, 1944, p. 297.

new goblet cells arise through a transformation of indifferent epithelial cells. . . . The transformation of a goblet cell into a common epithelial cell seems doubtful." Mucous exhaustion appears to signify a rather low-grade injury. It is commonly found at the borders of more severe changes (Fig. 5*B*).

Mucous necrobiosis of gland epithelia represents a more violent epithelial injury. It is found prominently in cases of short duration. It is characterized by loss of cellular polarity, by rounding-off of the cell body, and by multiple mucus-filled vacuoles which tend to fuse. The nuclei thus become compressed against the cell wall, with signet ring cells resulting (Figs. 2*B*, 3*A*, and 3*C*). Mucous necrobiosis appears to be a manifestation of irreversible injury of the epithelium and a profound deviation of cellular polarity and functional differentiation.

The most characteristic single features of the colitis are, first, surface exudation ("simple" pseudomembrane) and, second, stromal (diphtheritic) necrosis. Surface exudation and stromal (diphtheritic) necrosis are neither identical nor do they necessarily occur together.[10]

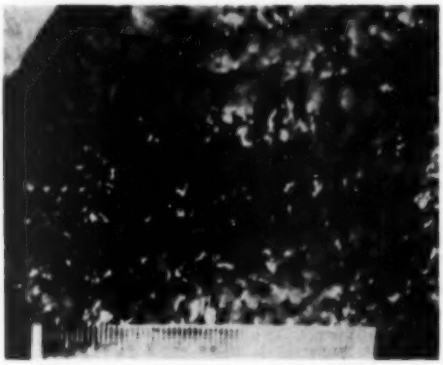

Fig. 1 (Case 1).—Confluent pseudomembranes in the ascending colon are moist and soft. This photograph was prepared from a kodachrome * transparency (scale in mm.).

In the very early stages of evolution some of the plaques are composed chiefly of mucus and epithelia with only few polymorphonuclear leucocytes and virtually no fibrin (mucoepithelial pseudomembranes[11]). The mucus may have been pro-

10. We are using the term "pseudomembranous inflammation" in the sense of O. Lubarsch (Entzündungen, in Aschoff, L.: Pathologische Anatomie, Ed. 7, Jena, Gustav Fischer, 1928, Vol. 1, pp. 567-571), thereby characterizing the colitis by its most prominent *gross* feature. It is appreciated, however, that on the microscopic level a given pseudomembrane may be due to surface exudation (croupous, W. C. MacCallum [A Textbook of Pathology, Ed. 2, Philadelphia, W. B. Saunders Company, 1922, p. 229], Lubarsch), or to stromal necrosis (diphtheritic, Lubarsch), or to both ("compound" pseudomembrane). The point is that in order to qualify, a pseudomembrane does not require a substantial amount of necrotic stroma. At any rate, there is no uniformity of nomenclature on the part of authors of various textbooks. Some of the modern American texts give the subject a rather illiberal treatment.

11. In a strict histopathologic sense the mucoepithelial pseudomembranes herein described belong really in the class of catarrhal inflammation. There can be no doubt, however, that they constitute the immediate precursors of fibrin-containing pseudomembranes (Siegmund[8]; MacCallum[10]).

duced by goblet cell hyperactivity (Fig. 2*A*), by muconecrobiosis (Figs. 2*B* and 3*A*), or by both. Internal coherence, as well as adherence to the mucosa, appears to be an expression of increased physical tenacity of the mucus. Between mucous casts, epithelia are discharged in vertical columns and bars, ghostlike derivatives of the gland pattern of the mucosa (Fig. 2*A*). Commonly, however, surface exudation (fibrin and a variable number of polymorphonuclear leucocytes) contributes materially to the plaques already in these early stages. The minimal prerequisite for its outpouring appears to be the denudation and blurring of the basement membrane (Fig. 2*B*). These "simple" pseudomembranes of surface exudate may be designated as mucoepithelial (Figs. 2A and 3A), fibrinomucoepithelial (Figs. 2*B* and 10*A*), fibrinopurulent, or mucofibrinopurulent (Fig. 8), depending on the preponderance of their constituent parts.

The stromal (diphtheritic) necrosis commences with decreased definition and impaired staining of stromal fibers and cells, aided perhaps by edema and some outpouring of fibrin (Fig. 2*B*). Although this early change is widespread, it does not usually reach deeply. Later on, the stromal (diphtheritic) necrosis becomes more profound. It appears as a closely knit spongework of deeply eosinophilic, swollen fibers enclosing in its meshes the residues of necrotic cells (Fig. 5*B*). Descriptively and without prejudice as regards the question of component fibrin, the appearance of this spongework may be referred to as "fibrinoid reticulum." Preexisting structures, such as vessels and basement membranes, are mere shadows, if recognizable at all. The necrosis blends quite imperceptibly into the surrounding viable stroma without any demarcating zone of inflammatory cells. Although there is much chronologic overlapping, it appears that with the longer survivors there is a tendency for the stromal (diphtheritic) necrosis to extend down to (Fig. 5) and even beyond the muscularis mucosae while, at the same time, decreasing in its lateral dimensions.

The degree of stromal (diphtheritic) necrosis determines the character of the adherence of the surface exudate. Characteristically, it is the interglandular summits which become the first sites of fibrin exudation and such necrosis; the resulting pseudomembrane thus becomes tagged onto the mucosa by microscopic-sized "pinpoints" (Fig. 10*A*). The deeper the stromal (diphtheritic) necrosis, the broader the adherence of the pseudomembrane, incorporating not only destroyed glands (Fig. 5*A*) but also fibrin pouring out from the depths of the gland crypts (Fig. 4*A*). In the longer survivors the pseudomembranes may become exceedingly small and assume a mushroom shape (Fig. 8*A*).

With destruction of the basement membrane and with increasing stromal (diphtheritic) necrosis, the latter blends, more or less imperceptibly, with the surface exudate. As the result of dehydration and fusion of the fibrin strands, the internal structure of the surface exudate comes to have a look which Councilman likened to the staghorn inlay of his pocket-knife. It appears compact and dry and resembles very much the "fibrinoid reticulum" of the stromal (diphtheritic) necrosis (Fig. 11*A*). Surface exudation and stromal (diphtheritic) necrosis having become fused, it may be difficult or impossible to recognize histologically what part of a given pseudomembrane relates to the former and what to the latter (Fig. 6*A*). This fused aggregate, too, appears macroscopically as a pseudomembrane and might advantageously be referred to as a "compound" pseudomembrane (Figs. 4*A* and 5*A*)

in contrast to the "simple" pseudomembrane composed predominantly of surface exudate.

In the mucosa, even in the early cases, the cellular infiltrate is made up largely of lymphocytes and plasmacytes (Figs. 2 and 3*B*). Macrophages become increasingly more numerous later. Eosinophilic leucocytes are scarce or absent at all times. Hemorrhagic diapedesis (Figs. 3*C* and 10*B*) is rarely a feature, and usually it is lacking altogether. Edema is quite variable in its incidence, its distribution, and its intensity (Figs. 2*B*, 10*B*, and 11*A*). Inflammatory infiltration is not restricted to sites of surface exudate or stromal (diphtheritic) necrosis. Polymorphonuclear leucocytes are not a significant constituent of the infiltrate in the tela propria and usually are entirely absent. However, they are selectively attracted to sites of acute denudation of the basement membrane subsequent to disruption of epithelial continuity and make their way into gland lumina (Fig. 3*B*) and into the surface exudate (Figs. 4*B* and 8*B*).

Cellular infiltration and edema are not confined to the mucosa. Edema may be prominent and even excessive in the submucosa (Fig. 6*B*). There is, in the early cases, associated heavy interstitial fibrin precipitation as well as acute lymphangitis. Also, and in contrast to what pertains to the mucosa, polymorphonuclear leucocytes constitute initially an appreciable percentage in the make-up of the cellular infiltrate in the submucosa. The submucosal hyperemia of the early phases is soon followed by prominent endothelial proliferation. From the submucosa inflammatory manifestations extend, in decreasing intensity, into the remaining coats of the colon, preferably but not exclusively along vessels.

Hyaline thrombi occupying mucosal venules are observed rarely and only in the longest survivors. Since they are not necessarily associated with infarct-type necrosis of the mucosa, it is inferred that their occurrence is a complication of stromal (diphtheritic) necrosis and, further, that the stromal (diphtheritic) injury is not on a thrombotic basis.

The lymphoid tissue of the mucosa does not react to any extent. No enlargement and no reaction centers become discernible. However, lymphoid follicles may, by contiguity, become involved from neighboring stromal (diphtheritic) necrosis and inflammatory infiltration. Reticulum cell proliferation is seen in later stages. These observations are analogous to those in regional lymph nodes, which, likewise, show reticulum hyperplasia but no appreciable reactivity on the part of the lymph follicles.

The nervous plexuses of Auerbach and Meissner may show interstitial edema and occasionally even inflammatory cell infiltration.

Reparative processes are observed early. The epithelium proliferates vigorously on almost any available surface, even on a necrotic one (Fig. 6*A*). Morphologic variations of the regenerated epithelia are quite striking and seem to depend on location, length of disease, type and extent of preceding epithelial injury, etc. The newly formed cells exhibit irregular spacing as well as varying degrees of dimensional and configurative atypy. They are quite flat, though broad-based when spreading on recently denuded surfaces, including those of glands (Figs. 4 and 10*A*). Later on, they are more voluminous, though still exhibiting lack of polarity, variation in size, and trigonal, square, or polygonal contour (Fig. 11). Their cytoplasm is pale bluish pink in hematoxylin-eosin preparations and varies from seeming

glassiness to hydropic swelling with peripheral condensation and exaggerated cell borders (Fig. 10*B*). The nuclei occur singly and vary in size and shape as well as in quantity and distribution of nucleoplasm (Fig. 10*B*). Although atypical, the nuclei are neither bizarre nor excessively large. They may have rather prominent eosinophilic nucleoli. There are no syncytial or multinucleated epithelia.

Mitotic figures are found mainly in the mature epithelia of gland crypts, accounting satisfactorily for regeneration in a centrifugal, i. e., upward, direction. However, there are glands lined entirely by the atypical cells of regeneration (Figs. 4*A* and 10*B*), and there is neoformation of glands in sites where there were no glands before (Fig. 8*A*). This may denote centripetal regeneration as well, i. e., new formation of epithelia extending from mucosal surface sideways and downward (Siegmund [3]). The dearth or absence of mitotic figures in regenerated epithelia can be interpreted to indicate amitotic division or diminished shedding or both. Also, the cells of regeneration seem to possess a remarkable degree of ameboid flexibility enabling them to compensate by alteration of shape what they lack in number. It is particularly the sidewise stretching at the expense of cell height which augments the mooring surface of the regenerating epithelium and thus enhances the efficiency of the relining of raw surfaces. Eventually the regenerated epithelium becomes polarized and approaches a more and more normal appearance.

Gland dilatation is both rare and mild. It is ascribable to mechanical blocking of the gland ostia, which in the earlier phases (Fig. 5*A*) is due to masses of surface exudation (mucus, fibrin). In the later stages it results from obstruction of gland necks, due either to narrowing of their over-all diameters or to hydropic swelling of regenerated epithelia or to both (Fig. 11*B*).

Healing proceeds via sequestration of stromal (diphtheritic) necrosis and surface exudation, and reepithelization keeps pace with their progressive shedding (Figs. 4*A* and 6*A*). Persistent ulcers, longitudinal arrangement along taeniae coli, undermining, mucosal bridges, or mucosal polyps are not observed. In other words, the disease is unlike iodiopathic ulcerative colitis.

The structural end result of pseudomembranous colitis will largely depend on the extent and the degree of the associated stromal (diphtheritic) necrosis. The more superficial the process, the more difficult will be the recognition of a preceding colitis. The sites of "simple" pseudomembranes with no or only little stromal (diphtheritic) necrosis (mucocellular, Figs. 2*A* and 3*C*; fibrin-containing, Figs. 2*B* and 9*A*) probably leave no permanent trace. Conversely, the deeper the diphtheritic component, the thinner will be the resulting mucosa. The mucosal pattern will be affected by numerical decrease as well as by altered course, length, and diameter of the glands (Fig. 10). This depends on the degree of preceding destruction of glandular basement membranes, particularly of that in the crypts. Profound atrophy and fibrosis will result from sequestration of deep stromal (diphtheritic) necrosis which has reached beyond the muscularis mucosae. There will be great dimensional and numerical diminution of glands, and those which re-form may come to rest in a fibrosed submucosa (Fig. 8*A*).

The newly formed lining of the colon is not resistant to the same injury which caused the original damage. Repeated necrosis (Fig. 10*B*), including pseudomembrane formation (Fig. 8*A*) as well as repeated reepithelization, may be demonstrated in it.

The small intestine was grossly congested in two of the six autopsy cases. In one case microscopic examination revealed a diffuse mononuclear cell infiltration of the mucosa and submucosa as well as swelling of vessel endothelia. There was no disorderliness to the epithelium. Another case showed focal enteritis, largely healed.

The esophagus and stomach were uniformly unremarkable in the gross; histologic sections are not available. The oral mucosa was taken as normal in six of the seven cases on the strength of clinical observation.

REPORT OF CASES

The following report of cases includes the five in which the patients had received "significant" doses of aureomycin or chloramphenicol. The order of presentation follows the severity of the colitis as measured particularly by the depth of stromal (diphtheritic) necrosis. This coincides largely with the length of survival after initiation of antibiotic therapy. Two further cases are appended to illustrate certain clinical and pathologic features: Case 6, in which the patient did not die and in which only the rectosigmoid became available for study as a surgical specimen, and which more than any other convinced us of the validity of our suspicion that aureomycin is causally related to the type of colitis in question; Case 7, in which the autopsy added strength to a similar suspicion with respect to chloramphenicol.

CASE 1 (A-49-127).—A white woman, 52 years of age, had undergone abdominoperineal resection of the rectosigmoid seven years ago. There were two subsequent revisions of a colostomy because of stenosis of the stoma, which leaked "copious amounts of mucus." She was poorly nourished and showed cheilosis. The hemoglobin content was 13.9 gm. The white blood cell count was 7,300. A barium sulfate enema given through the colostomy revealed no abnormalities of the colon and particularly no stenosis.

After three days of preparatory peroral aureomycin medication (3.5 gm. of the hydrochloride [12]) the colostomy was revised and a new stoma established. There was no colitis in the resected specimen. Aureomycin continued to be given (1.5 gm.), by intravenous route, together with 2.4 million units of penicillin, intramuscularly, until death. Abdominal distention and pain were complained of on the third postoperative day. No diarrhea was observed through the colostomy. Death occurred four days after the operation.

At autopsy, the colon was tremendously dilated, yet its wall was not thinned. Throughout the mucosa there were raised, grayish-white, firmly attached plaques. There was a decreasing gradient of this change, with respect to amount, size, and confluence, from the proximal to the distal colon. The diameters of the plaques ranged from 0.1 to 0.2 cm. in the distal colon and from 0.3 to 0.5 cm. in the more proximal portions (Fig. 1). The plaques were bordered by hyperemic halos. The terminal ileum presented lymphoid hyperplasia and congestion of the mucosa.

In the microscopic sections the gland epithelium shows either mucous exhaustion (Fig. 2*A*) or, more often, muconecrobiosis (Fig. 2*B*). The mucosa exhibits some mucoepithelial plaques (Fig. 2*A*), usually unassociated with stromal (diphtheritic) necrosis. More numerous are fibrinomucoepithelial pseudomembranes with more or less stromal (diphtheritic) necrosis (Fig. 2*B*). Polymorphonuclear leucocytes transmigrate focally at sites of denudation of basement membrane (Fig. 3*B*).

In the small intestine the tela propria is so densely infiltrated with lymphocytes and particularly with macrophages that there is bulbous thickening of the villi. Venules and thin-walled vessels show endothelial swelling and polymorphonuclear arrest. There is increased but orderly

12. In this, as in all subequent case histories, the doses stated are totals for the periods indicated. Irrespective of the route of administration, the patients of Cases 1 and 5 received aureomycin hydrochloride unbuffered whereas the remaining patients were given aureomycin hydrochloride buffered with sodium glycinate.

mitotic activity in the epithelium. In one section there is overproduction of tenacious mucus. There are no pseudomembranes and no stromal (diphtheritic) necrosis. The submucosa is loosely infiltrated with histiocytes.

Stool cultures were made on the fourth day after initiation of aureomycin medication. They revealed no enteric pathogens, but much growth of Proteus vulgaris, Pseudomonas aeruginosa, and Aerobacter aerogenes. No Escherichia coli was demonstrated.

Summary.—A surgical patient received 3.5 gm. of aureomycin hydrochloride perorally and 1.6 gm. intravenously over a combined total period of seven days. Colitis developed after more than three but in less than seven days. The pseudomembranes were closely set and frequently confluent, and were associated with only superficial stromal (diphtheritic) necrosis.

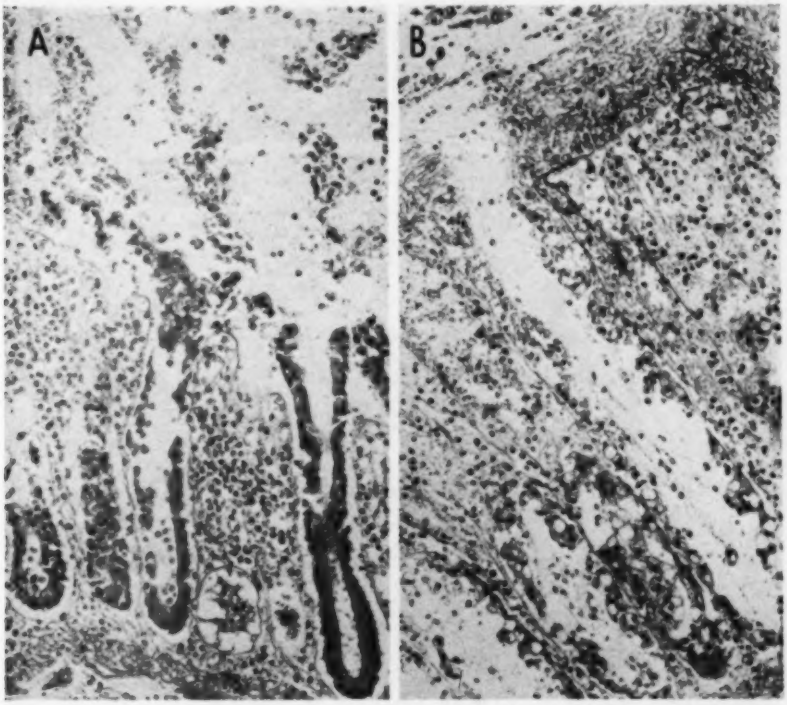

Fig. 2 (Case 1).—*A*, simple mucoepithelial pseudomembrane. Columns of desquamated epithelia and casts of mucus retain a ghost-like replica of the *in situ* gland pattern. There is mucous exhaustion of gland epithelia; the mucus on the surface must have been produced by preceding goblet cell activity. Polarization of epithelial cells is maintained. There is no stromal (diphtheritic) necrosis or fibrin exudation. The basement membranes are preserved, yet the continuity of gland and surface epithelium is patchily disrupted. There is a diffuse infiltrate of small mononuclear cells in the tela propria. × 225.

This and all subsequent photomicrographs are from sections fixed in 10% formalin (4% formaldehyde) and stained with hematoxylin and eosin.

B, simple mucofibrinoepithelial pseudomembrane. The gland lumen is filled with dense mucus although the ostium is not plugged. Note the superficial stromal (diphtheritic) necrosis and focal blurring of basement membrane. There is granular edema of the stroma. Polymorphonuclear leukocytes are absent. The crypts of Lieberkühn are lined with epithelia which are undergoing mucous necrobiosis and there is focal piling-up of epithelia. × 225.

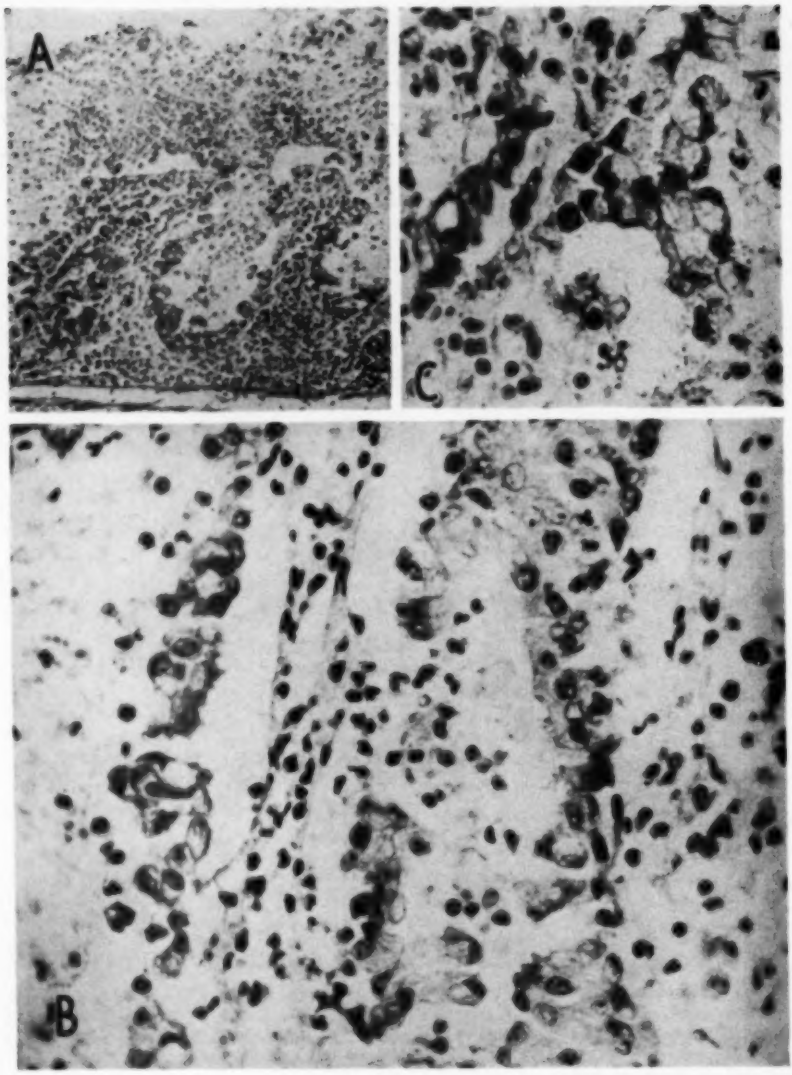

Fig. 3 (Case 2).—*A*, simple mucocellular pseudomembrane, adherent by sheer tenacity of the mucus. There is mucous necrobiosis of gland epithelia. There is no stromal (diphtheritic) necrosis. The tela propria is infiltrated by mononuclear cells. × 50.

B, tela propria infiltrated by lymphocytes, plasmacytes, and some macrophages. Polymorphonuclear leukocytes have been selectively attracted to foci of denudation of the basement membrane. The epithelium shows loss of polarity, degeneration, and occasional mucous necrobiosis. Essentially identical pictures were seen in Case 1. × 356.

C, signet ring cells resulting from mucous necrobiosis of gland epithelia. The uni- and multilocular vacuoles stained light blue with hematoxylin. Red blood cell diapedesis was occasionally found but rarely denser than here. × 356.

CASE 2 (A-50-63).—The patient, a white woman aged 72, was mildly diabetic. Six months earlier a diagnosis of rectal carcinoma was made. She entered the hospital because of tenesmus, rectal bleeding, and rectal serous discharge. Her nutritional state was poor. The hemoglobin content was 12.2 gm. The white blood cell count was 7,500. Stool examination gave essentially normal results.

After three days of preparatory intestinal "sterilization" with orally administered aureomycin (3.5 gm. of the hydrochloride), abdominoperineal resection was carried out. No pseudomembranous colitis was found in the resected specimen. Aureomycin (7.5 gm.) was administered subsequently by vein, until death. Penicillin, 3.6 million units, intramuscularly, was added during the last two days of life. The colostomy was left open but no stools were discharged until the seventh postoperative day. They were then diarrheic in character. Death occurred on the ninth postoperative day.

At autopsy the transverse colon was distended. Pseudomembranous colitis which decreased in intensity and confluence from proximal to distal portion was found throughout the entire length of the colon. In the descending and sigmoid segments the plaques, 0.2 to 0.4 cm. in diameter, were discrete, slightly raised, yellowish gray, and surrounded by erythematous halos. When scraped off, they left fairly smooth surfaces. Between the plaques the mucosa appeared normal. In the proximal portions of the colon the lesions were larger and more closely set. There was much confluence in the cecum, leaving very little uninvolved mucosa. In these areas of confluence the plaques were more difficult to wipe off and left a rough and congested surface. The appendix was obliterated by fibrosis. The small intestine was normal.

Microscopically, the lesions bear great resemblance to those of Case 1. The plaques range from mucoepithelial (Fig. 3*A*) to fibrinomucoepithelial to fibrinopurulent. Mucous necrobiosis with signet-ring cells (Fig. 3*C*) can be found unassociated with stromal (diphtheritic) necrosis (Fig. 3*A*), and inflammatory cell infiltration occurs in the tela propria without significant overlying epithelial changes. On the whole, there are less stromal (diphtheritic) necrosis and less fibrin outpouring than in Case 1. There is also more evidence of early healing. Sequestration of pseudomembranes is common. Hyperchromatic epithelial cells pile up in the gland bases and also on the mucosal surface underneath the sequestrated pseudomembranes.

Postmortem stool cultures revealed no enteric pathogens but showed much growth of Esch. coli and moderate growth of Pseudomonas aeruginosa.

Summary.—A surgical patient received 3.5 gm. of aureomycin orally, followed by 7.5 gm. intravenously, over a combined and continuous period of 12 days. Diarrhea was observed through the colostomy on the 10th day of medication. Pseudomembranes were closely set and confluent in the proximal colon and discrete in the distal one. Much healing of the pseudomembranous colitis was evident.

CASE 3 (A-50-56).—An 80-year-old white woman had undergone cholecystectomy for cholecystitis and cholelithiasis four years before. This was followed two years later by periodically recurring painless jaundice, dark urines, light-colored stools, occasional chills and fever, nausea, vomiting, and right-upper-quadrant abdominal pain. There had been swelling of the abdomen and of the ankles one month prior to the present admission. Her nutritional state was poor. The tongue was beefy, smooth, and dry. There was jaundice. Spider angiomas were noted on the back and neck. The heart was enlarged; there were harsh apical and aortic diastolic murmurs. Liver and spleen were palpably enlarged. She had ascites and pitting edema of the legs and the sacral region. There was venous distention of the neck. The hemoglobin content ranged from 8.0 to 10.7 gm., with a drop to 5.8 gm. on the 17th day. The white blood cell count ranged from 7,100 to 15,000.

The patient was treated with chloramphenicol, perorally, for the first 10 days of hospitalization. Diarrhea began on the fifth day and persisted throughout the greater part of hospitalization. The stools, numbering as high as seven per day, ranged from liquid to mushy and contained gross mucus on occasion; the guaiac test indicated occult blood 1 out of 5 times. Penicillin was administered intramuscularly in two courses of 23 and 5 days' duration (13.1 million units). On the 30th day a single oral dose of aureomycin hydrochloride (0.25 gm.) was promptly vomited. (For this reason the patient is listed in the table as receiving "chloramphenicol" rather than as receiving "aureomycin plus chloramphenicol.") Death occurred on the 40th day.

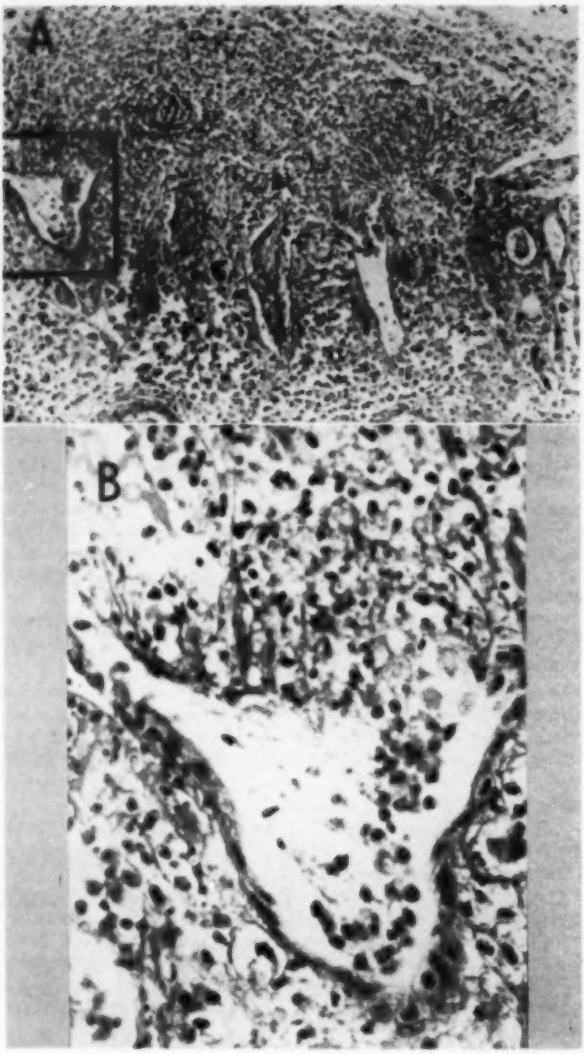

Fig. 4 (Case 3).—*A*, compound pseudomembrane. Surface exudate of staghorn pattern and "fibrinoid reticulum" of stromal (diphtheritic) necrosis blend into each other. Several interglandular summits are involved. Fibrin pouring from gland crypts joins the surface exudate. Note early sequestration of the compound pseudomembrane. The regeneration of gland and surface epithelium is characterized by a stretched-out character of the cells. × 80.

B, higher-power magnification of the framed area of *A*. It shows details of epithelial regeneration and of sequestration of pseudomembrane. Note polymorphonuclear leukocytes in the surface exudate. × 350.

At autopsy, gallstones were found in the main intrahepatic bile ducts. There were cirrhotic changes of the liver. The colon presented pseudomembranous inflammation, the foci often being as small as pinheads. They were slightly raised, centrally depressed, and usually located on the ridges of the mucosal folds. They were difficult to wipe off. There was no intestinal distention.

The microscopic sections of the colon show, in contrast to the preceding cases, that the stromal (diphtheritic) necrosis and the predominantly fibrinous surface exudate are more focal and circumscribed. Usually, no more than four to six interglandular stromal summits, and frequently no more than a single one, become involved in any one location and at any one time. In the more extensive examples fibrin exudes not only from the interglandular summits but from the gland crypts as well (Fig. 4*A*). The surface exudate is of typical staghorn type and blends imperceptibly with the "fibrinoid reticulum" of the underlying stromal (diphtheritic) necrosis. The stromal (diphtheritic) necrosis may come close to the muscularis mucosae. Epithelial regeneration (Fig. 4*B*) is confined to the periphery of stromal (diphtheritic) necrosis.

There is prominence of macrophages in the inflammatory exudate of the submucosa. There is a fair though not exclusive topographical correlation between the foci of mucosal necrosis and the density of submucosal cellular infiltration. Lymphangitis and vascular engorgement are less but endothelial proliferation is more striking than in Cases 1 and 2. The subserosa and the mesenteric fat are mildly infiltrated with chronic inflammatory cells.

Sections from the small intestine show no inflammatory changes.

Stool cultures, 26 days after initiation of chloramphenicol medication, revealed no enteric pathogens but much growth of Ps. aeruginosa. There was no Esch. coli. Postmortem cultures revealed no enteric pathogens but much growth of P. vulgaris and Aerobacter aerogenes. Esch. coli was not demonstrated.

Summary.—A nonsurgical patient received 30 gm. of chloramphenicol by mouth during the first 10 days of hospitalization. Diarrhea became established on the fifth day and continued nearly until death, more than a month later. There was low-grade fever throughout. The active stromal (diphtheritic) necrosis and surface exudation as late as 30 days after the drug had been discontinued illustrate the tendency of the disease toward self-perpetuation. The stools were still free of Esch. coli at autopsy.

Case 4 (A-50-27).—A white man, 79 years of age, had long-standing angina pectoris and Parkinson's syndrome, both on an arteriosclerotic basis. There had been complaints of constipation for many years. Hernioplasty was performed five years ago. Benign prostatic hypertrophy developed with urinary tract infection, one year ago. He was admitted to the hospital because of severe epigastric burning and pain, with loss of consciousness of several hours' duration. He appeared well developed and well nourished. The heart was enlarged to the left, with distant sounds and an apical systolic murmur, Grade 2. The rigidity of the abdomen was attributed by the neurologist to Parkinson's syndrome. Intestinal peristalsis was active. There was bilateral costovertebral tenderness. X-ray examination revealed no evidence of intestinal obstruction. The hemoglobin content was 12.7 gm. The white blood cell counts ranged from 8,700 to 14,000.

Aureomycin hydrochloride (totaling 6.1 gm.) was administered intravenously in two separate courses, from the 1st to the 3d and from the 7th to the 11th hospital days. During the second course, an additional 1.5 gm. of aureomycin was substituted perorally for a period of one day. Diarrhea began on the 5th hospital day, i. e., two days after the first intravenous course had been terminated; it recurred two to three days after the beginning of the second course. From then on, diarrhea, up to six stools per day, persisted until shortly before death. Penicillin (1.7 million units) was administered intramuscularly from the 15th to the 20th day. The body temperature was about 101-102 F. throughout hospitalization. The patient died on the 24th day.

At autopsy, the colon presented pseudomembranous colitis, which was least in the transverse colon and which increased in intensity both proximally and distally. Plaques, approximately 0.2 cm. in diameter, were difficult to scrape off. Between the plaques the mucosa was covered with orange-colored mucus, which could be removed easily and which was most profuse in the transverse colon.

In the microscopic sections the lesions reveal a decided tendency to remain discrete and small (Figs. 5*A* and 6). The mucosa impresses by its hilly relief (Fig. 6*B*). In the valleys there is total goblet cell transformation and also mucous hyperproduction (colica mucinosa). In

contrast, the crests exhibit mucous exhaustion of the epithelium and highly circumscribed foci of stromal (diphtheritic) necrosis involving as little as a single interglandular summit. The stromal (diphtheritic) necrosis often extends deeply and may obscure the muscularis mucosae (Fig. 5). It may occur without (Fig. 5*B*) or with fibrinous surface exudate of the staghorn pattern (Fig. 6*A*). Reparative changes, i. e., epithelial regeneration and sequestration of pseudomembranes, are similar to those observed in the preceding cases (Fig. 6*A*).

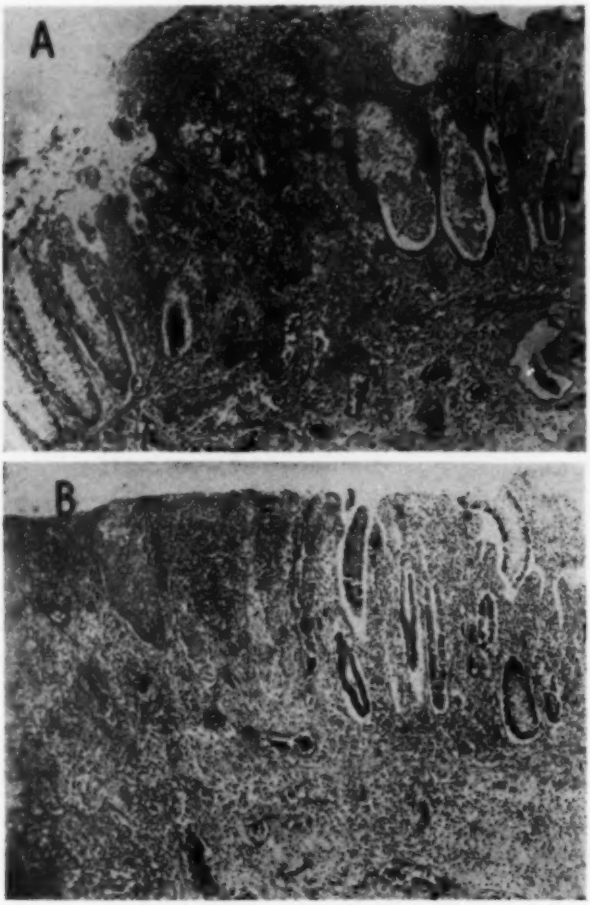

Fig. 5 (Case 4).—*A*, compound pseudomembrane. A "fibrinoid reticulum" of stromal (diphtheritic) necrosis blends broadly and imperceptibly with a flat fibrinopurulent surface exudate of staghorn pattern. Note obstructive dilatation of two glands, which are lined with flat epithelial cells indicative of early regeneration. The muscularis mucosae is obscured. Note discreteness of the mucosal necrosis. There is congestion of submucosal vessels. × 30.

B, pure stromal (diphtheritic) necrosis patterned as a "fibrinoid reticulum" without surface exudation—to the left. Associated muscularis mucosae is not recognizable. The glands near the stromal (diphtheritic) necrosis are in a state of mucous exhaustion. × 30.

The submucosa is excessively edematous, more so than in any of the other cases examined, often measuring as much as 0.3 to 0.5 cm. in thickness (Fig. 6*B*). Cellular infiltration is particularly severe underneath stromal (diphtheritic) necrosis (i. e., in mucosal crests) and is qualitatively and quantitatively like that found in the mucosa; particularly numerous are macro-

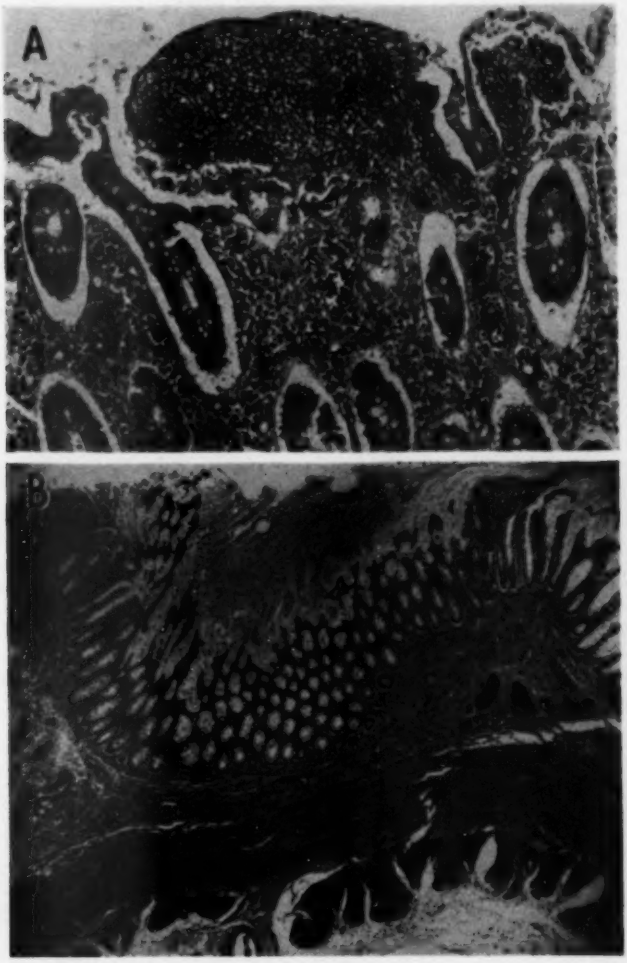

Fig. 6 (Case 4).—*A*, discrete pseudomembrane of staghorn pattern, which is being sequestrated. On the right the level of epithelial regeneration suggests that the pseudomembrane is solely the result of stromal (diphtheritic) necrosis; on the left, solely the result of surface exudation. × 80.

B, transverse colon. Note the hilly relief of the mucosa. In the valleys there is hyperproduction of tenacious mucus. On the crests there is circumscribed stromal (diphtheritic) necrosis; on the right this is associated with surface exudate. The edema of the submucosa is conspicuous but confined to mucosal ridges. × 15.

phages extending as far out as the serosa. There is some mild focal hemorrhagic diapedesis. The Meissner plexuses are edematous.

Stool cultures, nine days after initiation of the first course of aureomycin therapy, revealed no enteric pathgens. There was a moderate showing of coliform bacteria. The Gram-positive cocci were not identified (Streptococcus faecalis?). Two days later no enteric pathogens were shown. Ps. aeruginosa and coliform bacteria were present. Next day there were no enteric pathogens. There was moderate growth of coliform bacteria and P. vulgaris.

Summary.—This was a nonsurgical case and the patient the only male in the series. He was also the one patient in whom pseudomembranous colitis developed on intravenous rather than on peroral aureomycin medication. Aureomycin was given in two separate courses of three and five days' duration, respectively. Anatomically, pseudomembranous patches alternated with mucosa the seat of total goblet cell transformation of the glands and mucous hyperproduction (colica mucinosa). Stromal (diphtheritic) necrosis frequently occurred without surface exudation and occasionally extended beyond the muscularis mucosae. The case again illustrates the observation that the colitis was self-perpetuated for extended periods (two weeks) after termination of antibiotic therapy. The preference of stromal (diphtheritic) necrosis and surface exudation for the crests of the mucosa suggests that the pathogenic agent resides in, and acts from, the lumen of the colon.

Case 5 (A-49-110).—A white woman, aged 72, had rheumatoid arthritis of 15 years' duration. Fourteen years ago she had undergone cholecystectomy. Seven months prior to admission, following an episode of severe constipation, a barium sulfate enema revealed diverticulosis of the descending and sigmoid portions of the colon. In the distal part of the transverse colon there was an area of questionable blurring of the mucosal pattern, 10 cm. in length.

The woman was poorly nourished. Pemphigus vulgaris had been present for six weeks. This involved wide portions of the integument as well as the mouth, the nose, the scalp, and the vaginal orifice. There was no history of vomiting, diarrhea, or abdominal pain. The abdomen was soft, symmetrical, and not tender. There were rheumatoid deformities of multiple joints. The hemoglobin content varied between 9.5 and 12.2 gm. The white blood cell count rose steadily from 7,500 to 41,500. The patient was treated with large doses of aureomycin, given perorally in two courses from the 1st to 6th hospital day (9.75 gm. of the hydrochloride) and from the 9th to the 28th day (39.5 gm.). There was no diarrhea, but the guaiac test of the stools for occult blood was positive during the terminal two or three weeks. Death occurred on the 39th day.

At autopsy, the colon was not distended. Its mucosa appeared thin and atrophic but not fixed to the underlying coats. It was covered with irregularly spaced, discrete (0.2 to 0.6 cm.) and confluent yellowish pseudomembranous plaques, which decreased in number from the cecum to the sigmoid (Fig. 7). There were numerous diverticula.

In the microscopic sections the mucosa is frankly atrophic, often aglandular, and focally fibrosed. Not infrequently, the muscularis mucosae is not recognizable, and the intestinal glands seemingly lie in fibrosed submucosa (Fig. 8*A*). Throughout the colon there are discrete (Fig. 8*A* and *B*) and confluent pseudomembranes. Muconecrobiosis of glandular epithelium is found occasionally. Fibrinomucopurulent pseudomembranes are particularly numerous in the sigmoid and are clearly superimposed on areas previously diseased (Fig. 8*A*). There are, in the ascending colon, mucosal foci of infarct-type necrosis which involve sites of glandular muconecrobiosis, mucofibrinous pseudomembranes, or both. Infarct-type necrosis is always associated with clusters of occlusive hyaline or fibrinoid thrombi occupying mucosal venules of the particular focus. It never extends deeper than the muscularis mucosae. The smaller foci, in particular, have a roughly triangular shape, with the base directed toward the lumen. In spite of the venous location of the thrombi there is no interstitial hemorrhage; localized acute inflammatory reaction is either absent or only relatively mild.

Elsewhere and unassociated with infarct-type necrosis one can demonstrate an occasional mucosal or submucosal venule containing occlusive or nonocclusive hyaline thrombi.

A section through the ileocecal valve demonstrates the rigid confinement of the mucosal and mural inflammation to the colon in that the terminal ileum is entirely free of it.

Stool cultures were not made.

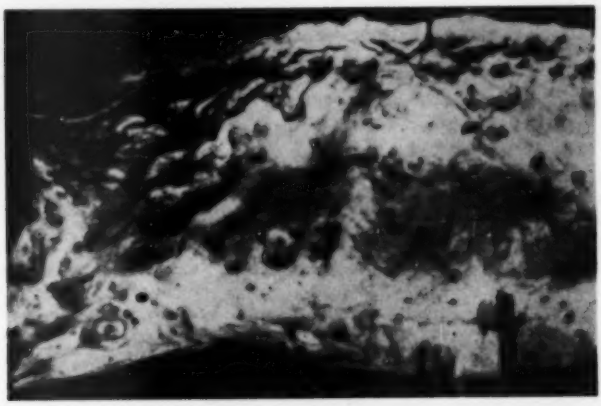

Fig. 7 (Case 5).—Descending colon, formalin-fixed. On an atrophic mucosa there are discrete, round or irregular pseudomembranes, which are distinctly yellow, quite dry, and firm.

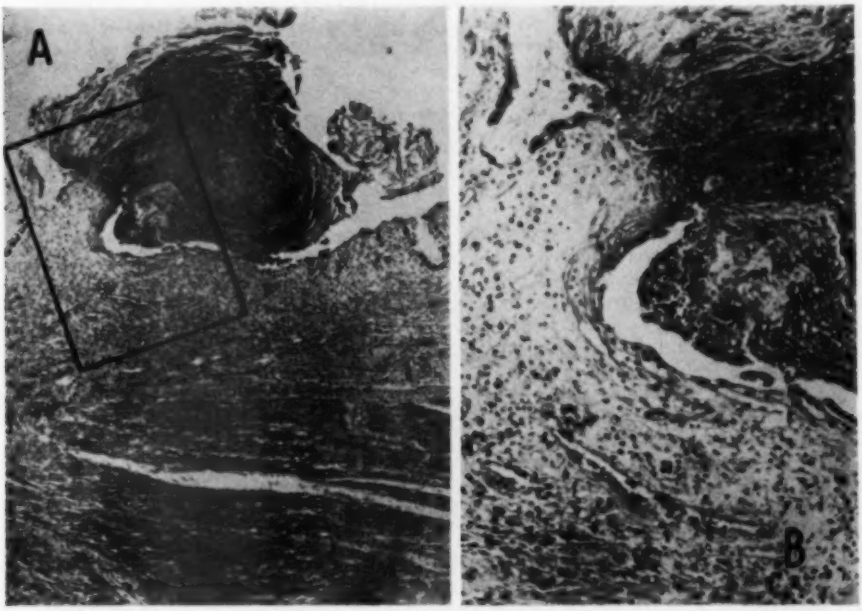

Fig. 8 (Case 5).—*A*, end-result of deep stromal (diphtheritic) necrosis. The lumen of the colon is lined by an epithelized, fibrosed, and hypoglandular layer probably corresponding to the original submucosa. The layer is moderately infiltrated by cells of chronic inflammation. The glands at the right appear to be entirely newly formed at sites where there were no glands before. A discrete pseudomembrane of mushroom shape projects above the surface, evidence of recurrent pseudomembranous colitis. × 30.

B, high-power magnification of the framed area of *A*. Polymorphonuclear leukocytes have been selectively attracted into the pseudomembrane. × 80.

Summary.—This nonsurgical patient was one of the longer survivors (39 days) and the one receiving the largest dose of aureomycin (almost 50 gm. of the hydrochloride over a total period of 26 days). Yet, there was no diarrhea. Several stools were guaiac-positive. There was much scarring of the intestinal lining; nevertheless, even the scarred "mucosa" remained subject to further surface exudation and stromal (diphtheritic) necrosis. There were focal thrombotic phenomena in venules and small veins with mucosal foci of infarct-type necrosis.

CASE 6 (S-50-3409).—A white woman, 68 years of age, attempted suicide by swallowing 3 oz. (90 cc.) of a proprietary disinfectant containing "neutral oils obtained from coal tar, cresylic acid, soaps, and 10% water." Two hours later the patient's stomach was lavaged. She had hematomas of the tonsillar fossae, petechiae of the palate, and white plaques on one of the vocal cords. No further ill effects of the poison were noted. Treatment was then directed to a carcinoma of the colon, knowledge of which had led to the suicidal attempt. There was no proctitis on sigmoidoscopy. The x-ray report after a barium sulfate enema was: "Colon negative except for encircling carcinoma of the rectum. No evidence of obstruction." Preparatory to surgical treatment of the bowel, aureomycin hydrochloride (19 gm.) was given for 12 days; administration of this antibiotic was discontinued because of spiking temperatures and thrombophlebitis of the legs. Instead, 20.5 gm. of streptomycin was given for the next 11 days. Also, the patient received, in two separate courses of 11 and 15 days' duration, respectively, 52.5 million units of penicillin intramuscularly. Very shortly after the resumption of peroral aureomycin medication, one week after this had been discontinued, violent diarrhea developed, which had been absent until then. Rectal examination now revealed the carcinoma to be very tender in contrast to the findings three weeks earlier. Two days after resumption of aureomycin medication (3.5 gm.) the patient was operated on (combined abdominoperineal resection of the rectosigmoid). The subsequent clinical course was characterized by abdominal pain and poor color of the exposed colostomy bowel. Aureomycin medication was continued, intravenously, (5.0 gm.) for six days following the operation when diarrhea recurred through the colostomy. The diarrhea was not convincingly controlled by folic acid given for a period of two weeks (10 mg. per day). The patient was discharged 33 days after the operation.

The surgical specimen was a 33-cm. rectosigmoid bearing a plateau-shaped carcinoma measuring 7 by 8 cm. in its flat dimensions (*c* in Fig. 9*A*). The surface of the tumor was coarsely rugated and broadly folded and varied in color from the dirty green of surface necrosis to angry red where sloughing had occurred (Fig. 9*B*). The carcinoma had penetrated the wall of the colon. There was no distention of the rectosigmoid proximal to the tumor. The proximal portions of the specimen were characterized by boggy edema, which coarsened the transverse folds. Rather than occurring in discrete plaques, the pseudomembrane was diffuse and of tissue-paper thinness in the proximal portion (*a* in Fig. 9*A*). It involved equally the crests of the edematous folds and the intervening crevices and could be wiped off readily with one's finger, exposing a finely pitted velvety base. The process extended to the surgical cut edge and decreased in a caudad direction. In the region of the tumor and a few centimeters proximally (b_x in Fig. 9*A*) the mucosa appeared quite normal. Distal to the carcinoma (*d* in Fig. 9*A*) the rectal mucosa was lightly peppered with discrete plaques appearing as elevated, intensely yellow, guttate elevations, 0.1 to 0.2 cm. in diameter (Fig. 9*B*). They bore no relation to lymphoid follicles. They were more firmly adherent than the confluent pseudomembrane of the proximal portions of the specimen. There was no gross edema distal to the neoplasm. The perirectal lymph nodes were not enlarged.

In the microscopic sections, proximal to the carcinoma (*a* in Fig. 9*A*) the mucosa is covered with pseudomembranes which are composed mainly of fibrin enmeshing mucus, epithelia, and some cells of acute and chronic inflammation (Fig. 10*A*). In general, the sites of attachment are microscopic "pinpoints" and are characterized by extremely superficial stromal (diphtheritic) necrosis with focal loss of covering epithelium. What mucus is mixed with the fibrin appears to have been contributed by previous goblet cell hyperproduction rather than by muconecrobiosis; at any rate, the glands are now in a state of mucous exhaustion. There are early sequestration of the pseudomembranes and coincident epithelial regeneration. In a few instances a more deeply reaching "fibrinoid reticulum" of stromal (diphtheritic) necrosis involves one-half of

the mucosal thickness; this is sometimes associated with fibrinous surface exudate of the stag-horn pattern (compound pseudomembranes).

Although normal on gross inspection, the mucosa near the carcinoma (b_1 b_2 and d in Fig. 9*A*) exhibits evidence of healed colitis. Changes in density, course, depth, and diameter of the reconstituted glands indicate incomplete reconstruction (Fig. 11). Fairly recent epithelial regeneration extends into the glands even as far down as the crypt bases (Fig. 11*B*). The tela

Fig. 9 (Case 6).—*A*, resected rectosigmoid colon (surgical specimen). There is bogginess of the mucosa (a, b_1, b_2) especially at and above the level of the carcinoma. (c) There is diffuse pseudomembrane in the most proximal portion of the specimen, involving the line of surgical resection. Letters indicate the sites of microscopic sections.

B, ampulla recti and distal portion of carcinoma corresponding to areas c and d of *A*. Note discrete, elevated pseudomembranes surrounded by erythematous halos.

propria appears fibrosed (Fig. 11*B*); there may be stainable edema of its subsurface portion (Fig. 11*A*). The inflammatory infiltration is not dense and consists mainly of lymphocytes and plasmacytes. In several foci the regenerated epithelium of surface and glands is again eroded, calling forth a circumscribed acute inflammatory infiltration and a hemorrhagic diapedesis

(Fig. 10*B*). The submucosa shows only few inflammatory cells, although edema is often considerable.

As though by mechanical protection, the best preserved normal mucosa of any of the sections is found beneath the overhanging edge of the carcinoma.

The lymph nodes of the perirectal and mesenteric fat show considerable "sinus catarrh." There is a rather striking reticulum cell hyperplasia within the lymphoid tissue as well. The follicles have no reaction centers.

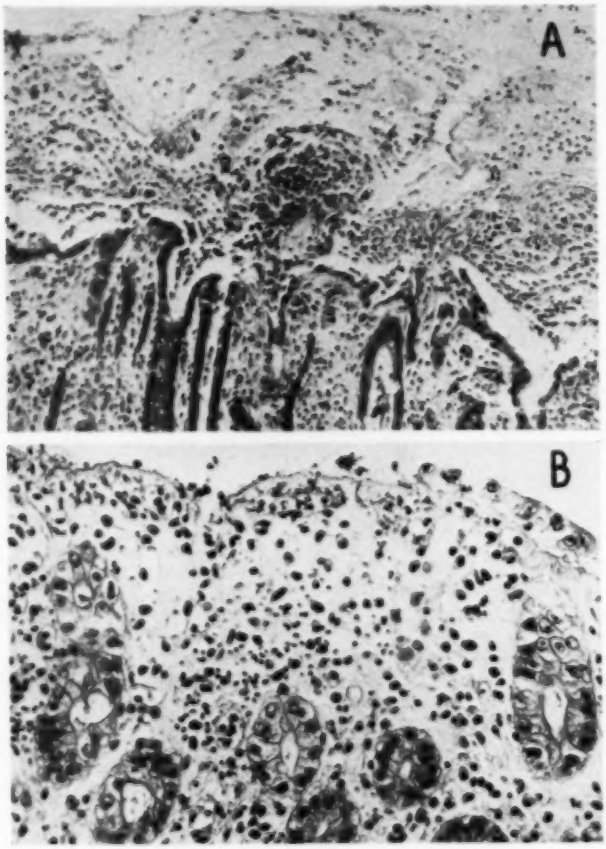

Fig. 10 (Case 6).—*A*, section from area *a* of Figure 9*A*. Simple fibrinomucoepithelial pseudomembrane with multiple "pinpoint" attachments to interglandular summits. There is hardly any stromal (diphtheritic) necrosis. Some of the glands are narrow and lined by the compact, deeply staining epithelium of mucous exhaustion. The mucosal surfaces are being covered over with flat epithelial cells of regeneration. There is incomplete sequestration of "pinpoint" attachments. × 75.

B, section from area b_3 of Figure 9*A*. Acute erosion is recurring in previously injured mucosa. The epithelium of the surface and the glands is quite atypical. Note the superficiality of the acute inflammatory reaction. There is red cell diapedesis. The gland epithelia exhibit exaggerated cell borders and variation of nuclei in size, shape, and density. × 225.

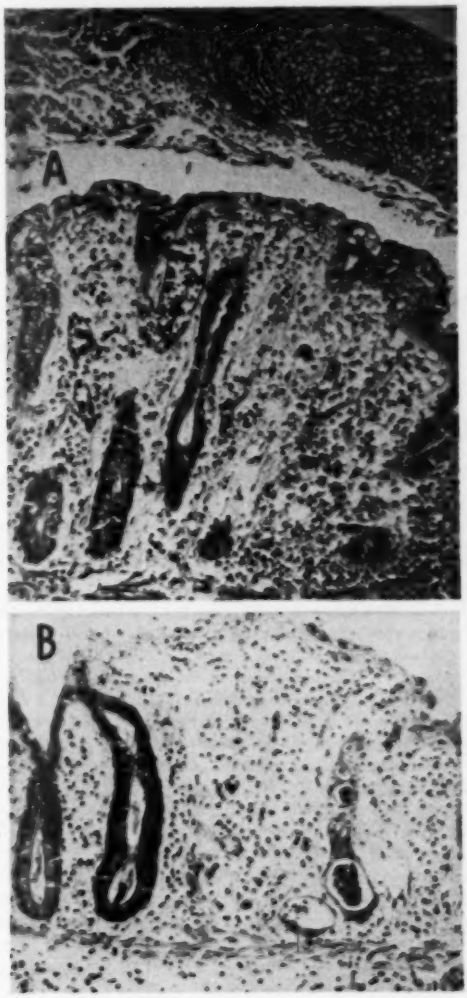

Fig. 11 (Case 6).—*A*, section from area *d* of Figure 9*A*. It shows a predominantly fibrinous pseudomembrane of staghorn structure. Its "pinpoint" attachment is in a plane slightly different from that of the section. There is a numerical decrease of glands, as well as a slight alteration of their course. The surface of the mucosa and some gland necks are lined with flat, cuboidal, or polygonal cells. Polarization is completed in the gland bases. The tela propria is edematous. × 75.

B, section from area b_1 of Figure 9*A*. Note the irregular distribution, the altered course, and the diameter of the glands. The stroma is fibrosed. There are columns of trigonal and polygonal cells of regeneration with hardly a gland lumen. One crypt is cystically widened and lined with exceedingly flat regenerated cells. The maximal gland diameter is not increased above normal and is actually decreased in the neck portion. There is edema of the submucosa. × 75.

Stool cultures were made from the surgical specimens on inhibitory and noninhibitory media, inoculated directly from colonic mucosa. No enteric pathogens were shown. A few anaerogenic paracolon organisms, A. aerogenes, and a moderate growth of Staphylococcus albus were observed.

Summary.—By the time of operation the patient had received, in two separate courses, 21.5 gm. of aureomycin hydrochloride perorally and 1 gm. intravenously. As the result of the first course, pseudomembranous proctitis developed, with stromal (diphtheritic) necrosis. The residue of this was represented, at the time of operation, by regenerated epithelium associated with mild to moderate structural distortion of the mucosa (b_1, b_2, d in Fig. 9*A*). This indicated that proctitis need not cause diarrhea, tenesmus, etc. The active pseudomembranous colitis (a in Fig. 9*A*) proximal to the carcinoma probably coincided with the development of diarrhea following a second course of aureomycin medication. The distribution of the lesions indicated, therefore, ascending spread. The exquisite freedom from any damage, past or present, shown by the mucosa beneath the overhanging edges of the carcinoma suggested that the injurious agent resides in and acts from the lumen of the intestine. The case also illustrates the probability that the regenerated epithelium is not immune to repeated injury by what is presumably the original agent.

Case 7 (A-51-28).—A white woman, aged 54, had a history of congestive heart failure, intermittent for three years, and had been treated with digitalis. Her temperature was slightly elevated throughout the hospital stay. An electrocardiographic tracing showed left bundle-branch block. The tongue was red; otherwise her nutritional state was satisfactory. The hemoglobin content was 14.3 gm. The white blood cell count was 10,700. A large peptic ulcer was demonstrated on the lesser curvature of the stomach by x-ray.

On the day after admission the patient was started on peroral chloramphenicol therapy. Because of the onset of diarrhea this therapy was discontinued after three days and after 4.5 gm. had been taken. There were eight stools per day for two days. On the 13th day a course of peroral aureomycin treatment (15 gm. of the hydrochloride) was begun and continued until the 20th day in spite of diarrhea (three to four stools per day) lasting from the 15th to the 26th day. A second course of peroral aureomycin therapy (9.5 gm.) given between the 30th and 38th day failed to produce diarrhea. Penicillin (5.4 million units) was administered intramuscularly from the 5th to the 13th day. The guaiac test of the stools was negative on seven occasions.

After two and a half weeks, dermatitis of the perianal and vulvar regions developed, followed, one month later, by stomatitis. Both were interpreted as being due to aureomycin therapy, and the patient was treated with methylrosaniline chloride, N. F. The lesions were not cultured, but moniliasis was suspected on clinical grounds. Death occurred on the 52d day from congestive failure and low salt syndrome.

At autopsy, the small and large intestines, particularly the transverse colon, showed foci of severe congestion but grossly no pseudomembranes.

The microscopic sections of the colon reveal mucofibrinoepithelial pseudomembranes. These are exceedingly flat and are associated with stromal (diphtheritic) necrosis of variable depth. The glands exhibit varying degrees of epithelial degeneration and disruption of continuity. There are extensive fresh petechiae, confined to the mucosa. Patchy scarring of the mucosa is present together with a numerical decrease of the glands. There are multiple occlusive thromboses of venules, confined to the superficial portion of the mucosa, which, unlike those in Case 5, are unassociated with infarct-type necrosis.

In the small intestine the changes are few but definite. Several small foci are demonstrated where the mucosa is simplified, avillous, hypoglandular, slightly edematous, and infiltrated with moderate numbers of mononuclear and polymorphonuclear inflammatory cells. The reconstituted Lieberkühn's crypts are lined with slightly atypical epithelial cells, which are cuboidal, irregularly spaced, and poorly polarized. The mucosal inflammation extends into the immediate subjacent submucosa, accentuating focally an otherwise mild though diffuse exudation of this coat. Elsewhere, the mucosa is the seat of fresh petechiae.

Stool cultures were not made.

Summary.—This nonsurgical patient illustrated the identity of clinical and presumably also of pathologic manifestations following, respectively, chloramphenicol and aureomycin medication. The two drugs were given in independent courses, and diarrhea followed in two of the three

courses. This patient demonstrated again that anatomic colitis may be present without diarrhea (the second aureomycin course). Venule thromboses of the mucosa were similar to those of Case 5 but, unlike those, were not associated with infarct necrosis. This was the only case of the series in which orificial skin lesions developed. These clinically resembled moniliasis and possibly were caused by Candida albicans. This was the only case presenting permanent mucosal injury of the small intestine.

COMMENT

Of the seven patients with colitis, only five had diarrhea after antibiotic medication had been instituted. Thus, while diarrhea was a frequent development, not all the patients who presented pseudomembranous colitis presented this symptom. Furthermore, the diarrhea was not of even intensity throughout the course of the colitis.

Intravenous as well as peroral administration of aureomycin can produce diarrhea and pseudomembranous colitis.[13] The one patient (Case 4) who received aureomycin by vein received, for one day, a small amount of the drug by mouth; yet diarrhea was well established long before the oral intake. Also, two patients (Cases 1 and 2) receiving aureomycin by mouth preoperatively did not have diarrhea, nor did their surgical specimens show pseudomembranous colitis. In these same patients a few days of postoperative administration of aureomycin, given intravenously, caused full-blown pseudomembranous colitis.[14]

The pseudomembranous colitis under discussion recapitulates the striking female sex preponderance which has been observed with all types of side reactions, including diarrhea. For instance, in Harris'[18] series of 135 patients treated with aureomycin or chloramphenicol the ratio was approximately 3:1 in favor of women. He surmised "that sex hormones played some role, perhaps through interference with enzymatic activity." Seed and Wilson[15] found that oral administration of aureomycin produces consistently higher blood levels of the antibiotic, and maintains them for longer times, in women than in men. They speculated that this might be brought about by differences of absorption, or of utilization, or of excretion. The authors favored the absorption hypothesis because, on simultaneous intake of aluminum hydroxide, which adsorbs the antibiotic to a great extent, this sex difference largely disappears.

The two antibiotics may act synergetically with adjunct factors. Intestinal distention, for instance, was invoked even in the preantibiotic era as a cause of colitis (stercoral diphtheria, intestinal diphtheria).[5] Similarly, postoperative states in general[4a] and postoperative shock in particular[4b] have been implicated in the causation of pseudomembranous enterocolitis. While these conditions might have been factors in the surgical cases, 1, 2, and 6, they were clearly not in the nonsurgical remainder of this series. The patients in the latter not only failed to show any significant degree of colonic distention on radiographic, clinical, or anatomic examination but also failed to exhibit any manifestations of shock.

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13. Jacob, S.; Schweinburg, F. B., and Rutenburg, A. M.: Effect of Intravenous Aureomycin on the Intestinal Flora of Dog and Man, Proc. Soc. Exper. Biol. & Med. **78**:121, 1951.

14. More recently one of us (G. M. M.) observed several patients who had diarrhea after exclusively intravenous aureomycin medication. There were also roentgenographic changes in the colon.[2]

15. Seed, J. C., and Wilson, E. C.: The Effect of Aluminum Hydroxide on Serum Aureomycin Concentration after Simultaneous Oral Administration, Bull. Johns Hopkins Hosp. **86**:415, 1950.

Florid pseudomembranous colitis was still present at the time of death in Cases 5, 4, and 3, respectively, 11, 13, and 30 days after termination of antibiotic therapy. This indicates that, once established, the disease has a tendency either toward self-perpetuation or toward slow healing.

The designation of certain factors as "significant" with respect to the development of colitis is based on the smallest values of drug dosage and duration of administration in our cases: As regards peroral administration of aureomycin, the patient in Case 1 had shown neither diarrhea nor colitis at the time we received the surgical specimen; up to then the patient had taken 3.5 gm. of aureomycin hydrochloride over three days. An additional 1.6 gm., by vein, over an additional period of four days, was productive of colitis. Therefore, the "significant" dose of the drug lay between 3.5 and 5.1 gm. and the "significant" time factor between four and seven days. With respect to peroral administration of chloramphenicol, if one assumes that diarrhea expressed the onset of pseudomembranous colitis, the patient in Case 7 had the disease with a total of 4.5 gm. of this antibiotic over a period of three days, while the patient in Case 3 required 12 gm. and a period between three and five days. Concerning intravenous administration of aureomycin, the patient in Case 5 showed diarrhea, and thus presumably pseudomembranous colitis, from 2.5 gm. of drug and after five days. Therefore, the "significant" values were taken as follows: survival of more than four days after the first dose of (*a*) at least 4.0 gm. of aureomycin hydrochloride or of chloramphenicol by mouth or (*b*) at least 2.0 gm. of aureomycin hydrochloride by vein. Pseudomembranous colitis cannot be considered a common complication of antibiotic medication, however, since 26 patients at autopsy (Table) did not exhibit pseudomembranous colitis in spite of their having received "significant doses" of either or both drugs.

It does not seem probable that the pseudomembranous colitis and diarrhea were due to impurities of the drugs. The colitis has followed not only fermentative aureomycin of increasing purity but synthetic chloramphenicol as well.

Cultures of stools of five of the seven patients were made during hospitalization. Because of the search for intestinal pathogens, such cultures were usually conducted on selective media. No enteric pathogens were demonstrated in any case. The known inhibitory influence of antibiotic treatment on Esch. coli and the overgrowth of such micro-organisms as P. vulgaris, Ps. aeruginosa, and A. aerogenes are illustrated in several of the cases. The presence (reappearance ?) of Esch. coli after 10 to 12 days of continuous aureomycin therapy (Case 2) has been observed,[16] as has been its prolonged absence after discontinuation of the therapy (Case 3). Staph. albus was identified in the one instance (Case 6) in which noninhibitory media were inoculated. No cultures were made for fungi. However, Gram-stained tissue sections of the colon did not reveal mycelia in any of the six colons examined, and only once were fungal spores found.

In some cases the guaiac test of the stools was positive for occult blood. In others the stools contained gross mucus. These data are incomplete and do not reveal any constancy of pattern or association. The body temperatures were more or less elevated during those periods in which colitis had become established, but one is

16. Marshall, H. C.; Palmer, W. L., and Kirsner, J. B.: Effects of Chemotherapeutic Agents on Fecal Bacteria in Patients with Chronic Ulcerative Colitis, J. A. M. A. **144**:900, 1950.

not certain of a causal relationship. In Case 6 the temperature dropped to normal immediately after removal of the sigmoid. Yet, because the line of surgical resection went through active pseudomembranous colitis, that part of the colon left behind was almost certainly the seat of additional disease. Leucocytosis was constant but of variable intensity, the usual count being between 10,000 and 15,000. Hemoglobin and red cell values exhibited no discernible pattern of alteration. Possibly related to antibiotic therapy was the clinically unexpected drop of hemoglobin level and red cell count in Case 3 five days after chloramphenicol treatment had been discontinued.[17]

Some of our patients showed evidence of nutritional impairment: others did not. The blood protein values were generally within normal limits. Also, generally, no mention is made of oral or skin lesions in the clinical or the autopsy records. Pseudomembranous colitis, therefore, can evolve in the absence of other undesirable side effects of antibiotic therapy.

There are several mechanisms by which aureomycin and chloramphenicol might conceivably induce pseudomembranous colitis. Although said to be generally of low toxicity, they may become injurious to the colon by some special direct or indirect drug action.

After peroral administration both aureomycin [18] and chloramphenicol [19] are readily absorbed from the upper gastrointestinal tract. Aureomycin is excreted in the urine and, to a lesser degree, in the bile. With utilization of bio-assay, urinary excretion can account for only 10 to 20% of the total administered dose of aureomycin.[20] The amount excreted in the bile is more obscure. It is stated that aureomycin is excreted in the bile in high concentrations.[21] Yet, in terms of percentage of total drug intake, probably no more than 5 to 10% would be expected, since biliary concentrations of aureomycin, as determined in bio-assay, are placed at only one-third to one-half of those found in the urine.[18] We infer that a large part, perhaps as much as 70% of the total intake of aureomycin, is not accounted for in bio-assay of the excreta.

In contrast, the excretory behavior of chloramphenicol has become amenable to chemical rather than bio-assay analysis. Glazko, Wolf, Dill, and Bratton [19] were thus able to show that in man 90% of the drug is excreted in the urine, largely in the form of bacteriologically inactive compounds. The bile contained an additional 2.7% and the stool about 1% of the total of inactive compounds. With bio-assay, only 10% of the administered chloramphenicol can be recovered in the urine.[19]

It is possible that aureomycin, too, is largely excreted in urine and bile in the form of biologically inactive metabolites. If such excretory metabolites were toxic

17. Volini, I. F.; Greenspan, I.; Ehrlich, L.; Gonner, J. A.; Felsenfeld, O., and Schwartz, S. O.: Hemopoietic Changes During Administration of Chloramphenicol (Chloromycetin), J. A. M. A. **142**:1333, 1950.

18. Herrell, W. E., and Heilman, F. R.: Aureomycin: Studies on Absorption, Diffusion and Excretion, Proc. Staff Meet., Mayo Clin. **24**:157, 1949.

19. Glazko, A. J.; Wolf, L. M.; Dill, W. A., and Bratton, A. C., Jr.: Biochemical Studies on Chloramphenicol (Chloromycetin): II. Tissue Distribution and Excretion Studies, J. Pharmacol. & Exper. Therap. **96**:445, 1949.

20. Dowling, H. F.; Lepper, M. H.; Caldwell, E. R., Jr.; Whelton, R. L., and Brickhouse, R. L.: The Concentration of Aureomycin in Urine and Cerebrospinal, Pleural and Ascitic Fluids After Oral and Intravenous Administration, J. Clin. Invest. **28**:983, 1949.

21. Jacob and others.[13] Herrell and Heilman.[18]

to the intestinal tract either intrinsically or after further intraintestinal conversion, it might account for the higher incidence of colitis in females: Since women absorb larger portions of orally administered aureomycin,[15] larger amounts of excretory metabolites should be expected in the bile and thus in the intestinal canal. It may also be significant that the only male patient of this series received aureomycin by vein, a route which again produces comparatively higher blood levels [22] and presumably more excretory metabolites. Conversely, if direct contact with the unchanged antibiotic, either unabsorbed or reexcreted, were the cause of pseudomembranous colitis, one should see more severe and more frequent intestinal changes in males than in females.

Of course, the two drugs may become injurious to the intestinal mucosa by being directly converted, within the intestinal canal, into toxic compounds. According to Crooks,[23] one should have expected chloramphenicol to be toxic because of its nitrophenol skeleton. Similarly, too, Seligman [24] points out that in the side chain of chloramphenicol,-CO.CH.Cl_2, chlorine is in the alpha position to a carbonyl group. Such chlorine is likely to be reactive and may inactivate certain sulfhydryl-bearing enzymes, an action similar to that of iodoacetate and the nitrogen and sulfur mustards. This is of particular interest because these mustards are known intestinal poisons which produce histopathologic pictures not entirely unlike those observed in our cases.[25]

Vitamin deficiencies have been incriminated most commonly for the various side reactions of antibiotic therapy. They should be considered in the pathogenicity of pseudomembranous colitis, particularly since colitis has been observed in association with several vitamin deficiency syndromes. There are important similarities, as well as differences, both of clinical and of pathological nature, between deficiency colitides and the pseudomembranous colitis of antibiotic therapy. No single feature appears pathognomonic for any deficiency colitis or, indeed, for the pseudomembranous colitis of antibiotic therapy. The repetition of various histopathologic features may indicate a limited reactivity on the part of the tissues. It may also mean that several pathogenic agencies act via a common denominator. In either case the differences of clinical and pathological behavior may be merely variations of such coincidental factors as time, intestinal flora, etc.

Similar to our patients, Denton's [26] pellagrous patients exhibited, in the colon, pseudomembranes, alterations of surface and gland epithelium, and numerical decrease of glands. Absent in our cases and present in Denton's were the uniform thickness of the colonic wall, the diffuse redness and smoothness of the mucosa, and the "small gray bodies" of mucus-retention cysts; neither did we observe the striking accumulations of eosinophiles, the telangiectasias of the submucosa, or the mucosal abscesses.

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22. Brainerd, H. D.; Bruyn, H. B., Jr.; Meiklejohn, G., and Scaparone, M.: Assay of Aureomycin in Body Fluids: Observations on Individuals Receiving Aureomycin, Proc. Soc. Exper. Biol. & Med. **70**:318, 1949.

23. Crooks, H. M., Jr., cited by Herrell, W. E.: Newer Antibiotics, Ann. Rev. Microbiol. **4**:101-128, 1950.

24. Seligman, A. M.: Personal communication to the authors.

25. Graef, I.; Karnofsky, D. A.; Jager, V. B.; Krichesky, B., and Smith, H. W.: The Clinical and Pathologic Effects of the Nitrogen and Sulfur Mustards in Laboratory Animals, Am. J. Path. **24**:1, 1948.

26. Denton, J.: The Pathology of Pellagra, Am. J. Trop. Med. **5**:173, 1925.

Experimental pantothenic acid deficiency of swine (Wintrobe and associates[27]) causes flecky to confluent exudates and ulcerations of the colon. The earliest histologic change is mucous exhaustion ("atrophy") of the gland epithelium. There are superficial ulceration, exudation of polymorphonuclear leucocytes, and progressive cystic dilatation of glands. The end-stages quite resemble pellagra colitis.[28] In their evolution and various phases of the pathologic picture these experimental cases differ from our cases.

Prolonged folic acid deficiency of monkeys is described as essentially an ulcerative process with purulent exudate in the gland bases.[29] Cystic glands lined with flat epithelia, as well as mucous exhaustion, are encountered in the non-ulcerated mucosa. The animals become anemic and leucopenic.

After folic acid analogues have been administered to humans, intestinal changes evolve as quickly as in our cases. They are epitomized by the Hass group[30] as intense, generalized, hemorrhagic, ulcerative colitis. Similar to our observations, the initial trauma appears to be to the epithelium. Mucus production ceases and goblet cells disappear. Disintegration of the epithelium is repaired by large, immature, and atypical cells. Cystic transformation of glands occurs. Small ulcers result from inefficient regeneration of the epithelium; they tend to spread and are covered with a "tenacious diphtheritic membrane." Obviously, there are similarities with our cases. We did not observe, however, the unduly large and atypical nuclei which are seen following administration of folic acid analogues.[31] Cystic dilatation of glands, furthermore, was rare in our cases and hemorrhagic manifestations were minimal or entirely absent. Moreover, our patients exhibited neither the peripheral anemia or leucopenia nor the bone marrow hypoplasia of that syndrome. Indeed, the bone marrows of our patients were, if anything, hyperplastic.

The unimpressive effects of the folic acid administered in our Case 6 do not necessarily speak against a relationship of this vitamin and pseudomembranous colitis due to antibiotics, since the toxic effects of folic acid analogues are also not influenced by folic acid administration.[32] Although not determinable in the case of aureomycin, the structural formula of which is unknown, it may be pointed out that at least chloramphenicol, by its structure, would not be expected to be a com-

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27. Wintrobe, M. M.; Follis, R. H., Jr.; Alcayaga, R.; Paulson, M., and Humphreys, S.: Pantothenic Acid Deficiency in Swine, with Particular Reference to the Effects on Growth and on the Alimentary Tract, Bull. Johns Hopkins Hosp. **73**:313, 1943.

28. Follis, R. H., Jr.: The Pathology of Nutritional Diseases, Springfield, Ill., Charles C Thomas, Publisher, 1948.

29. Rinehart, J. F., and Greenberg, L. D.: Colitis in the Folic Acid-Deficient Monkey with Notes on Similarities to Ulcerative Colitis in Man, Am. J. Path. **24**:710, 1948; Nutritional Concepts as Revealed by Studies of Thiamine, Folic Acid and Pyridoxin Deficiency in the Rhesus Monkey, Am. Pract. **4**:230, 1949.

30. Taylor, S. G., III.; Hass, G. M.; Crumrine, J. L., and Slaughter, D. P.: Toxic Reactions of 4-Amino-Pteroylglutamic Acid (Aminopterin) in Patients with Far Advanced Neoplastic Disease, Cancer **3**:493, 1950.

31. Landing, B. H.: Personal communication to the authors.

32. Franklin, A. L.; Stokstad, E. L. R., and Jukes, T. H.: Observations on the Effect of 4-Amino-Pteroylglutamic Acid on Mice, Proc. Soc. Exper. Biol. & Med. **67**:398, 1948. Philips, F. S.; Thiersch, J. B., and Ferguson, F. C.: Studies of the Action of 4-Amino-Pteroylglutamic Acid and Its Congeners in Mammals, Ann. New York Acad. Sc. **52**:1349, 1950. Jersild, T., and Mehlsen, S.: Aminopterin Therapy in Leukemia in Childhood, Acta Paediat. **40**:127, 1951.

petitive analogue of pteroylglutamic (folic) acid.[24] It is worthy of note that all our patients received, by peroral and parenteral routes, large amounts and a great variety of vitamin preparations, including liver extract, with no obvious preventive or curative effects on the pseudomembranous colitis. This observation does not exclude the possibility that aureomycin and chloramphenicol or their respective breakdown products may interfere, locally in the colon, with dietary, medicinal, or bacterially produced vitamins which are necessary for the well-being of the intestinal lining cells.[33]

Finally, the common denominator causing colitis may not be one of drug toxicity or vitamin deficiency but one which derives from the effects of the antibiotics on bacteria. Since many of the side effects of aureomycin and chloramphenicol medication take place on body surfaces exposed habitually to a mixed bacterial flora (moist skin, mouth, vagina, colon), the very effect of selective bacteriostasis of the antibiotics on such mixed flora might constitute a pathogenic agent. One is inclined to paraphrase Lourie,[34] who writes on the demographic impact of chemotherapy: "Like so many of the technical triumphs of civilization (chemotherapy) is only ostensibly and superficially an unalloyed blessing. While solving some of our worst problems, it helps to create new ones." From a broad biologic point of view, antibiotics must exert a profound disturbance of the age-old, homeostatic balance of the microbial world living on the mammalian mucous membranes and skin. Viewed biologically, symbiosis is a state of balance not only of the flora on body surfaces but of the tissues exposed to the metabolic processes of this flora as well. Conceivably, unbalancing the normal habitant flora of a given location should equally unbalance the tissues which are inherently part and parcel of that symbiotic world. The more sudden the quantitative and qualitative alteration of the bacterial flora, the more profound ought to be the necessary adaptive changes of the contacted epithelia. Furthermore, when compared with a broad-spectrum antibiotic, a narrow-spectrum antibiotic acting foremost against Gram-positive micro-organisms might put a different adaptive demand on the mucosa of the intestine than, for instance, on that of the mouth. This might explain why penicillin causes oral lesions similar to those of aureomycin and chloramphenicol while leaving the intestinal mucosa free of damage. Unfortunately, the complexity of the normal intestinal flora renders it difficult to obtain proof for such an assumption. Yet, Metchnikoff long ago made such a suggestion in connection with his lactic acid bacillus therapy. Inevitably, one or several micro-organisms in homeostatic balance in a symbiotic world as above conceived will, after suppression of one or several others sensitive to antibiotics, become out of balance and turn harmful by sheer quantity of their metabolites, or by some other mechanism.

Because fungal infections can be diagnosed histologically it can be stated that colonization of fungi and in particular of C. albicans is not causally related to the pseudomembranous colitis.[35] Furthermore, intestinal pathogens, in the customary meaning of the term, were not found in this condition.

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34. Lourie, E. M.: Chemotherapeutic Agents, Ann. Rev. Microbiol. **1**:237-262, 1947.

35. Warning Statement to be Included in Aureomycin Hydrochloride, Chloramphenicol and Terramycin Hydrochloride Labeling: Council on Pharmacy and Chemistry, J. A. M. A. **145**:1267, 1951.

CONCLUSIONS

A pseudomembranous colitis may follow aureomycin or chloramphenicol medication.

The mucosa of the colon is the seat of two characteristic changes usually, but not necessarily, occurring together: surface exudation ("simple" pseudomembrane) and stromal (diphtheritic) necrosis. The "simple" pseudomembranes may be mucoepithelial, fibrinomucoepithelial, fibrinopurulent, or mucofibrinopurulent. The stromal (diphtheritic) necrosis is an essential constituent of "compound" pseudomembranes. It varies in lateral extension and in depth.

Healing occurs by sequestration of the pseudomembranes and simultaneous epithelial regeneration. The deeper the stromal (diphtheritic) necrosis, the more distorted, atrophic, or scarred is the resulting healed mucosa. The colonic mucosa is subject to repeated injury by the original agent.

Pseudomembranous colitis follows aureomycin not only after oral but also after intravenous administration.

The incidence of the pseudomembranous colitis shows a female sex preponderance.

Neither intestinal pathogens nor fungi are demonstrated.

A variety of vitamins neither prevents nor cures the pseudomembranous colitis. The clinical manifestations are not characteristic. Diarrhea is common but not constant.

The mechanism by which aureomycin and chloramphenicol produce pseudomembranous colitis is obscure.

EXPERIMENTAL RACHITIS OF THE VERTEBRAL BODIES OF RATS

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ACCURATE histologic descriptions of the changes produced by experimental rickets in the tibia and the rib have been published.[1] However, descriptions of the microscopic changes produced by standard rachitogenic diets in the vertebral column are lacking.

Gross rachitic changes in vertebral bodies of experimental animals were noted by Boer, Arons, and van der Rijst [2] when the severe kyphosis produced paralysis of the hindlegs. Follis, Day, and McCollum [3] found kyphotic changes in the thoraces of rats kept on a diet extremely low in phosphorus and determined the degree of kyphosis by measuring the water capacity of the thoracic cage. Goyena and Castillo de Bonnevaux [4] confirmed the work of Boer, Arons, and van der Rijst and described in more detail the gross features of the rachitic deformity. Dodds and Cameron [5] also described the gross features of rickets involving the spinal column. Measurements of the vertebral column, from the base of the skull to the caudal end of the innominate bone, demonstrated that in normal rats the vertebral column grew steadily until the 12th week, with an average increase of 47 mm. in eight weeks. In rachitic rats it grew only 12 mm. in the same period.

Coleman, Becks, Kohl, and Copp [6] studied the ninth caudal vertebrae in rats maintained on a diet low in phosphorus but of normal vitamin D content. The animals failed to grow at a normal rate. When killed, they presented the histologic picture of rickets in the tibial epiphyses. In the vertebral bodies the changes were lack of growth and increase in thickness of the epiphyseal cartilages.

Descriptions of gross rachitic spinal deformities in children are numerous in the earlier literature of the subject. This material has been reviewed, and the

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From the Department of Pathology, University of Michigan.

1. Park, E. A.: Observations on the Pathology of Rickets with Particular Reference to the Changes at the Cartilage-Shaft Junctions of the Growing Bones, Harvey Lect. **34**:157, 1938-1939.

2. Boer, J.; Arons, P., and van der Rijst, M. P. J.: On a New Syndrome Consequent on Vitamin D Deficiency in Rats, Arch. néerl. physiol. **22**:594, 1937.

3. Follis, R. H., Jr.; Day, H. G., and McCollum, E. V.: Histological Study of the Tissues of Rats Fed a Diet Extremely Low in Phosphorus, J. Nutrition **20**:181, 1940.

4. Goyena, H., and Castillo de Bonnevaux, S.: Contribución al estudio de la cifosis raquítica experimental con paraplejia, de las ratas blancas, Arch. urug. med. **28**:152, 1946.

5. Dodds, G. S., and Cameron, H. C.: Studies on Experimental Rickets in Rats: IV. The Relation of Rickets to Growth, with Special Reference to the Bones, Am. J. Path. **19**:169, 1943.

6. Coleman, R. D.; Becks, H.; Kohl, F. V., and Copp, D. H.: Skeletal Changes in Severe Phosphorus Deficiency of the Rat: I. Tibia, Metacarpal Bone, Costochondral Junction, Caudal Vertebra, Arch. Path. **50**:209, 1950.

histologic features have been described by one of us.[7] In human vertebrae there is an increase in the width of the epiphyseal cartilage with an increase in number of cells in the columns of proliferating cartilage.

METHODS

Since none of the material covered in the earlier report was from patients with severe spinal deformities, an experimental study on rats was planned in the hope that the microscopic changes accompanying severe deformities could be studied.

Thirty-three rats of the Wistar strain, weighing from 40 to 60 gm., were fed the U.S.P. rachitogenic diet No. 2. Three rats were killed each week for the first eight weeks of the experiment and also after 12, 13, and 14 weeks. Controls, which had been fed the standard laboratory diet, killed at the same time, were littermates of the animals with which they were to be compared.

None of the animals presented any evidence of cord compression, but slight deformities of the vertebral column were seen in the latter weeks of the experiment.

Longitudinal blocks of the vertebral column and of a tibia from each animal were decalcified by the formic acid technique, and sections were stained with hematoxylin and eosin.

OBSERVATIONS AND COMMENT

The rachitic state is manifested by several changes in the vertebral bodies. The changes in the proliferating cartilage of the epiphysis are easily evaluated by measuring the width of the proliferating zone and by counting the cartilage cells in the columns of that area. In order to minimize the effect of local variation, five measurements and counts were made on each specimen, and the averages of these were recorded in the accompanying table as the values for that animal. The figures obtained for the three animals in each age group were averaged and used to construct Figures 1 and 2 showing differences in thickness of the cartilage plates and differences in the numbers of cells in the columns of the proliferating zones. In general, there was an overgrowth of the proliferating zones in the rachitic animals (Figs. 3 and 4). This agrees with the observations of Dodds and Cameron [8] on rachitic animals and with those of Coleman and his co-workers [6] on animals with phosphorus-deficiency rickets. This overgrowth was not observed, or was inconstant, in the animals killed in the first three weeks of the experiment. It is believed that these early inconsistencies were due to growth disturbances related to the rats' apparent dislike for the rachitogenic diet.

There were changes in the cartilage plates that did not lend themselves to measurement. In the older animals there was irregularity of provisional calcification with consequent destruction of cartilage cells; also, small capillary "bushes" were invading the epiphyseal plates. These changes were the same as those in the costochondral junction in early rickets, but not even the cases in which rachitogenesis was most advanced showed the extreme distortion of the columns of proliferating cartilage cells that is part of the picture of typical rickets at the costochrondral junction. The severity of rickets is dependent, in part, upon the rate of growth. Dodds and Cameron [8] pointed out that the rate of growth of vertebrae is proportion-

7. Hendrix, R. C.: Rachitic Changes in Vertebral Bodies, A. M. A. Arch. Path. **53**:174, 1952.

8. Dodds, G. S., and Cameron, H. C.: Studies on Experimental Rickets in Rats: I. Structural Modifications of the Epiphyseal Cartilages in the Tibia and Other Bones, Am. J. Anat. **55**:135, 1934.

Cell Counts and Thickness of Zones of Proliferation

Time, Weeks	On Rachitic Diet		Controls	
	Cells	Thickness, μ	Cells	Thickness, μ
1	13	183	12	220
	11	144	13	211
	13	144	13	220
Av.	12	157	13	217
2	10	154	14	173
	10	144	15	202
	14	144	15	144
Av.	11	147	15	173
3	15	144	12	154
	15	134	13	144
	14	163	14	144
Av.	15	147	13	147
4	15	154	14	144
	15	163	12	134
	15	163	13	106
Av.	15	160	13	128
5	15	173	12	115
	17	173	13	154
	15	144	13	96
Av.	16	163	13	122
6	15	134	12	115
	14	125	11	115
	15	163	12	115
Av.	15	141	12	115
7	15	134	12	125
	15	106	12	115
	16	163	12	96
Av.	15	134	12	112
8	22	230	10	96
	17	163	10	96
	16	154	12	105
Av.	18	182	11	99
12	21	154	9	86
	30	288	8	96
	28	250	11	86
Av.	26	231	9	89
13	15	115	8	86
	28	220	10	86
	32	336	10	77
Av.	25	224	9	83
14	19	106	8	67
	16	106	8	58
	19	144	8	96
Av.	18	119	8	74

ally slower than that of the long bones. For this reason rachitic lesions should not be expected to be of equal severity in all parts of the body.

Although the measurable changes of the epiphyses were not reliable until the animals had been fed the rachitogenic diet for a few weeks, earlier changes associated with rickets were demonstrable. By the end of the second week of the experiment the bony trabeculae of the experimental animals were broader than those of the controls and were invested by a heavy coat of osteoid tissue. In the control animals the osteoid tissue diminished rapidly with increasing age until it became almost imperceptible. In the experimental animals, however, the osteoid tissue

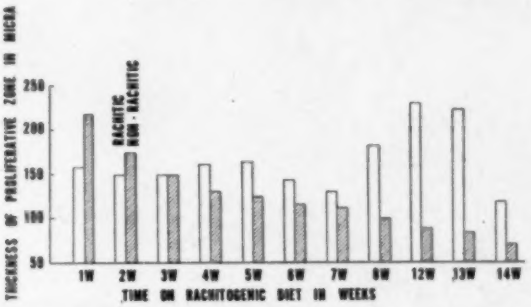

Fig. 1.—Graphic comparison of thicknesses of the proliferative zones of rachitic and nonrachitic vertebral bodies. The values used are the averages listed in the table.

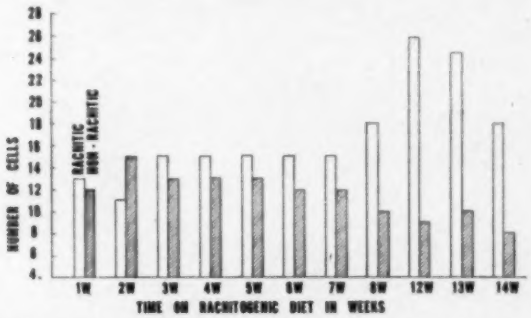

Fig. 2.—Graphic comparison of numbers of cells in the proliferative zones in rachitic and nonrachitic vertebrae. The values used are the averages listed in the table.

increased, at least relatively, with increasing severity of the disease. This feature was difficult to evaluate exactly after decalcification, but many preparations showed a distinct demarcation between osteoid tissue and calcified bone. In the more advanced stages of rickets the trabeculae lost their slender shape and longitudinal arrangement. This was a variable finding, with differences between individuals in the same time group and differences within the same animal. In general, the most marked distortion of the trabeculae was in the area immediately adjacent to the epiphyseal cartilage. Here, in advanced rickets, large masses of osteoid accumulated, leaving but little marrow space between them.

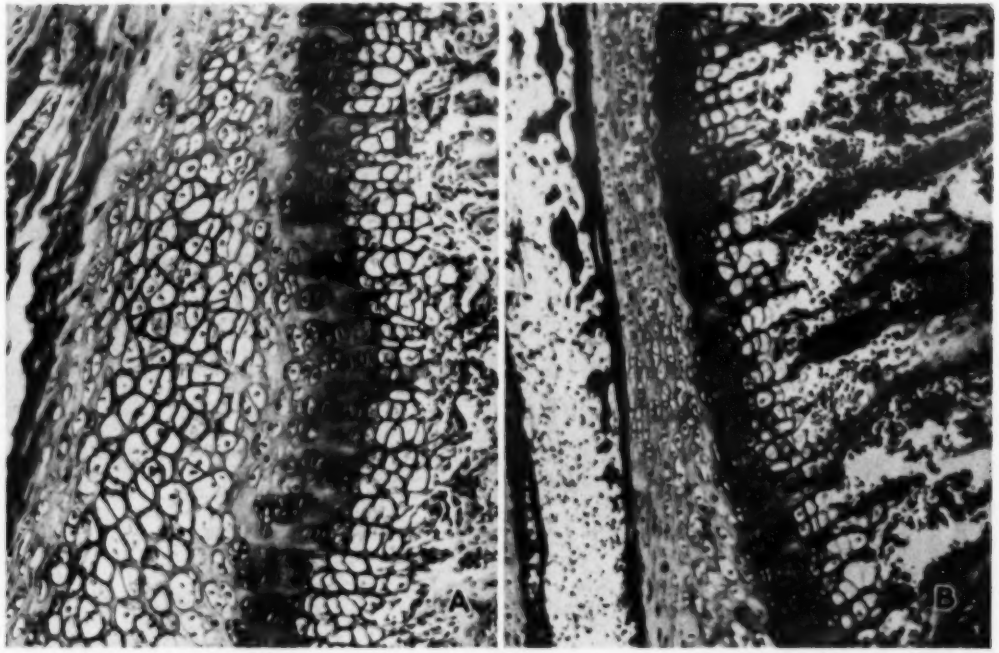

Fig. 3.—Cartilage plates of vertebral bodies from (*A*) a rat which had been restricted to a rachitogenic diet for three weeks and (*B*) a littermate which had been fed the standard diet. Hemalum and eosin stain; × 200.

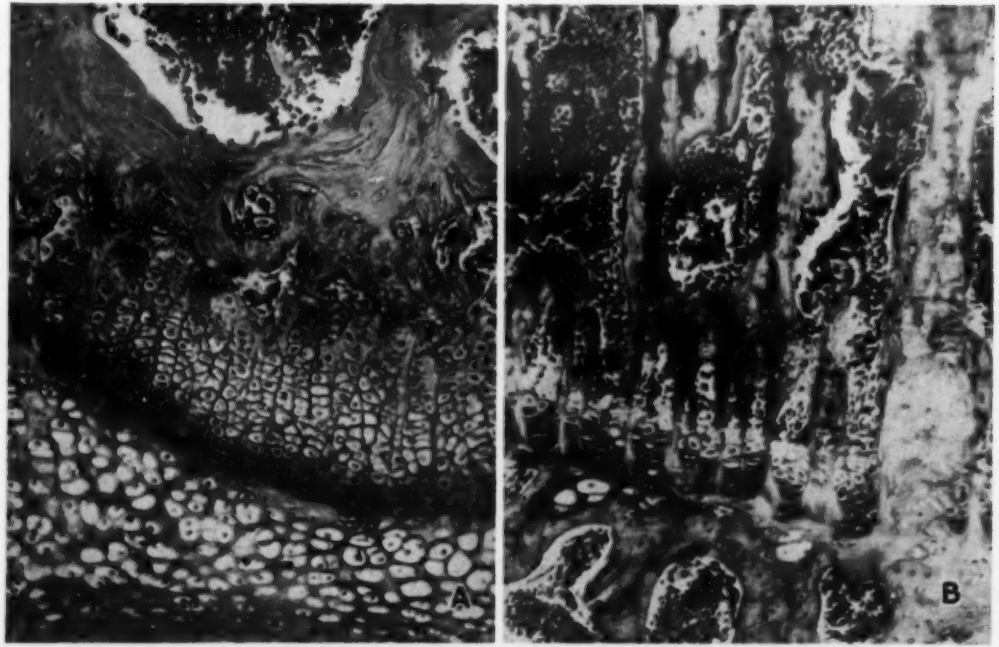

Fig. 4.—Cartilage plates of vertebral bodies from (*A*) a rat which had been restricted to a rachitogenic diet for 12 weeks and (*B*) a littermate fed the standard diet. Hemalum and eosin stain; × 200.

In the control material osteoblastic activity, as evidenced by the number of osteoblasts lining the trabeculae, diminished rapidly with increasing age. Although decreasing, it persisted much longer in the rachitic bones. The numbers of osteoblasts varied within each bone; they were more abundant at the growing ends of the bone and less abundant in the center. This was another manifestation of the slowness of growth produced by avitaminosis.

It was observed, also, that in animals which had been restricted to the rachitogenic diet for seven or more weeks, the vertebral bodies appeared to be relatively more slender than in the controls, and some of them were bent. In this group, also, the ends of the bones were widened. This feature did not lend itself to accurate measurement, since the plane of sectioning varied. These were evidences both of retarded growth and of softening of the poorly calcified bone.

The magnitude of measurable differences, as well as of the observable but unmeasurable differences, reached a peak at the 12th week. At this time, in the controls there was extensive ossification of the small bone-forming centers of the vertebral bodies, and very little growth activity persisted at the principal epiphyses. After the 12th week the measurable differences between rachitic and nonrachitic vertebral bodies began to diminish. The animals were approaching their limits of growth.

The diet used in this work was probably deficient in several essential food materials, and the animals did not take the diet well. At the end of the 14th week experimental animals weighed, on the average, 100 gm. less than the controls.

SUMMARY

In an attempt to extend and verify previous observations [7] on rachitic changes in human vertebral bodies, the disease was produced experimentally in rats. The essential findings were:

1. There is a definite increase in thickness of the epiphyseal cartilage with an increase in number of cells in the proliferating columns of cartilage in the rachitic bone.

2. Osteoid tissue is produced in greater abundance, and the poorly calcified trabeculae are distorted.

3. Osteoblastic activity is maintained at a high level in the rachitic material.

4. Gross structural deformities result, due to softness of the bone.

SO-CALLED "CAT SCRATCH FEVER"

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DURING the past few years, sporadic reports have appeared in the literature referring to a disease entity variously called "cat scratch fever," "cat claw disease," "la maladie des griffes de chat," "cat-bite fever," all of which dealt with a disease associated with localized lymphadenopathy, accompanied in most instances by a traumatic lesion (scratch) of the hand. In some instances the "scratch" was ignored, while in others no scratch was present and no history of trauma was elicited. As a rule, not until the regional lymph nodes enlarged was medical attention sought.

It is of especial interest that although these articles make some reference to contact with cats, the actual case reports do not consistently reveal such association.[1] Mollaret and associates [2] reported certain cases "where the cat could play no part," but they "found traces of scratches from rose tree, blackthorn, or bramble thorns." History of cat contact, with or without scratch, has preceded clinical evidence of lymphadenopathy in a sufficiently high proportion of cases to make one suspect this particular household pet. In all the reports of cases associated with cats, no recognizable illness of the cat is mentioned. Domestic cats have, on occasion, transmitted a number of diseases to man. These were listed and adequately discussed in a recent article by Greer and Keefer,[3] as follows:

Tularemia
Rabies
Rat-bite fever
Feline pneumonia (virus)
Diphtheria
Tuberculosis
Brucellosis
Typhus
Leptospirosis (?)
Ringworm (Microsporum felineum, Tinea circinata)
Creeping eruption (Ancylostoma braziliense)
Favus (Achorion schoenleini [now called Trichophyton schoenleini])
Pasteurellosis
Plague (?)
Histoplasmosis (?)
Cat fever

It is not the purpose of this report to establish the etiology of the disease or to throw light upon the vector. I would rather emphasize the morphologic aspects of the involved lymph nodes. During the past six years, four cases have come to my

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1. Thelin, F., and Martin-du-Pan, R.: New Observations of Disease Due to Cat Scratches, Praxis **40**:74, 1951.

2. Mollaret, P.; Reilly, J.; Bastin, R., and Tournier, P.: Nueva documentacion sobre la adenopatia regional subaguda y expontaneamente curable descrita en 1950, la linforeticulosis benigna de inoculacion, Presse méd. **58**:1353, 1950.

3. Greer, W. E. R., and Keefer, C. S.: Cat Scratch Fever: Disease Entity, New England J. Med. **244**:545-548, 1951.

attention. The first case (1946) was baffling to me and to a number of other pathologists, and sections of the enlarged axillary lymph nodes were interpreted as "early Hodgkin's disease," "lymphosarcoma with necrosis," "nonspecific inflammation," and "tularemia." There was no history of cat contact in this case, nor any local lesion of the hands. The next two patients each had sustained a recent cat scratch. The fourth patient did not have any known contact with cats. A brief summary of the cases follows.

REPORT OF CASES

CASE 1.—G. C., a 13-year-old white boy, was hospitalized on Aug. 15, 1946. Several days prior to admission, his mother noticed a "swelling" in the left axilla, of which the boy was unaware. The swelling was not painful or tender, and there were no associated symptoms. No history of recent trauma was elicited, and the boy had had no known contact with any domestic pets. Physical examination revealed a mass of matted nodes, about the size of a lemon, in the left axilla. They were slightly tender, but the overlying skin was not inflamed. Temperature, pulse, and respiration were normal. No other enlarged nodes were found, and the spleen was not palpable. A complete blood count and a urinalysis gave normal results. Agglutination tests for Brucella abortus, typhoid and paratyphoid Salmonellae, Proteus OX19, and Pasteurella tularensis were negative. The day after admission, the axillary mass was excised. The boy has remained well up to the present time (September, 1951), and there is no evidence of recurrent disease.

Pathologic Findings.—The specimen was an irregular, lobulated mass of enlarged lymph nodes enclosed in a fibrous capsule. On section there were two separate, similar groups of nodes, 4.0 and 2.0 cm. in diameter. The cut surfaces were studded with discrete yellow nodules up to 3.0 mm. in diameter, situated in a pinkish-tan stroma. The nodules pouted, and each one had a central core of pus.

Microscopically, the lymph node structure was distorted by large areas of epithelioid cells surrounding a central zone of nuclear debris and polymorphonuclear neutrophiles. Around the epithelioid cells there was a collar of lymphocytes mixed with mononuclear cells, reticulum cells, fibroblasts, and occasional eosinophiles.

CASE 2.—E. H., a 33-year-old white policeman, was well until approximately one week prior to hospitalization, when a painful swelling developed in the right axilla. He had daily chills and fever (temperature rising to 103 F.) for four to five days. When admitted to the hospital, he showed a granulomatous area at the base of the second finger of the right hand. He had been exposed to rabbit skins and a cat recently, and to "tuberculosis" for many years without known disease. Radiologic examination of the chest revealed no abnormality. Penicillin was given parenterally, and large red, indurated, painful areas appeared on the extensor surfaces of the arms and legs. The penicillin therapy was discontinued, and aureomycin was given. The joints became painful, and the wrists swollen. No other lymphadenopathy developed, and the spleen was not palpable. The fever gradually subsided, and the symptoms and skin lesions disappeared. Five days after admission, the axillary mass was excised. The wound healed promptly without sinus formation.

Laboratory Data.—The leukocyte count was 11,000 (differential count, normal); blood culture, negative; agglutnation tests for Brucella abortus, typhoid and paratyphoid Salmonellae, Proteus OX19, and Pasteurella tularensis, negative. Since discharge from the hospital (two years) the patient has been well.

Pathologic Findings.—The specimen was an irregular, enlarged lymph node, 2.5 cm. in widest diameter. The external surface was pinkish tan and lobulated. The cut surface was composed of small tubercle-like pouting yellow nodules situated in pale-tan stroma.

Microscopically, the structure of the node was distorted by moderately well-circumscribed foci of epithelioid cells surrounded by a collar of lymphocytes and mononuclear cells, plus an occasional Langhans-type giant cell. Within the ring of epithelioid cells there was a central core of polymorphonuclear neutrophiles mixed with fibrin and nuclear debris.

CASE 3.—G. K., a 68-year-old white man, was scratched by a domestic cat which had been in his household "for some time." About one week later, an ulcer appeared on the dorsum of the

left middle finger. The lesion oozed purulent material for approximately three weeks. The surrounding skin and subcutaneous tissues were not tender, reddened, or edematous, and there were no signs of cellulitis. However, for a period of approximately 10 days following the appearance of the ulcer the patient felt ill. Fever, chills, and sweating were absent. Concurrently with the appearance of the ulcer, he noticed several enlarged nodes in the left axilla, approximately the size of a cherry, which rapidly increased to tennis-ball size in about three days. The nodes were not particularly painful, and there was no associated redness or edema. He consulted his physician at this time, and received several doses of penicillin and "sulfa." The local finger lesion, as well as the axillary mass, failed to recede, and he was hospitalized approximately five weeks following the initial cat scratch. His past and family history were not contributory.

When admitted to the hospital, the patient appeared to be in no acute distress. His temperature ranged from 98.6 to 100.4 F.; the pulse rate was 80 to 90 a minute, and the respiratory rate was 20 a minute. The heart was enlarged slightly to the left, and there were no murmurs; the rate and the rhythm were normal. The blood pressure was 150/85. The lungs were clear to percussion and auscultation. The abdomen was slightly obese, but no masses or viscera were palpated. On the dorsum of the left middle finger there was a skin ulcer, 1.0 cm. in diameter, the edges of which were somewhat indurated, without redness or tenderness. The left arm was not edematous, and no limitation of motion was observed. The epitrochlear glands were not enlarged. In the left axilla, a firm spherical mass was found, approximately 6 by 3 by 3 cm. It was freely movable except for a single point, at which it was attached to the lower edge of the left pectoralis major muscle. The mass was slightly tender, and the surface felt lobulated.

The day after admission, the axillary mass was excised; it was accidentally ruptured in the process, releasing approximately 20 cc. of thick, yellow pus. The mass appeared to be a group of coalesced nodes. Three or four small nodes about the size of a bean were found adjacent to the larger mass, and these were excised also. During the excision, and after the purulent exudate had been released, the surgeon accidentally pricked his finger (no inflammation or ulceration resulted). The axillary wound healed slowly, and there was postoperative swelling of the left arm and hand, which gradually subsided over a period of a month.

Laboratory Data.—The results of urinalysis were normal. The hemoglobin content was 11.4 gm.; the red blood cell count, 3,800,000; the leukocyte count 11,000 (polymorphonuclear neutrophiles 62%, band cells 6%, lymphocytes 32%). Serologic tests were negative. The erythrocyte sedimentation rate (Wintrobe) was 30 mm. A culture of the axillary pus was sterile; guinea pig inoculation proved negative for tuberculosis. A tuberculin test and the Frei test were negative. Agglutination tests for typhoid and paratyphoid A and B Salmonellae, Brucella abortus, Proteus OX19, and Pasteurella tularensis were negative. Approximately two and one-half months after excision of the axillary mass, and three and one-half months after the original cat scratch, a skin test of the tuberculin type was performed with antigen supplied by Dr. Lee Foshay from another case of "cat scratch fever." The test was positive (4 plus) in 24 hours. At present (eight months later) the patient is well, without evidence of residual disease.

Pathologic Findings.—The specimen was a lobulated mass, 7 cm. in diameter, of enlarged, matted nodes. The cut surface consisted of circumscribed gray nodules, each with a tiny central area of pus. A large portion of the interior of the mass was replaced by a beefy red, coarsely granular necrotic zone, which was partly lined by a thin layer of purulent exudate.

Microscopically, the cytoarchitecture was largely distorted by single and confluent pseudotubercles, each of which showed a central zone of necrotic cellular debris mixed with polymorphonuclear neutrophiles, surrounded by a ring of epithelioid cells which fused with an outer zone of lymphocytes and mononuclear cells. Occasionally a Langhans-type giant cell was found at the periphery of a "tubercle."

CASE 4.—J. C., a 20-year-old white college student, was admitted to the hospital on July 2, 1951. Ten days before, he had a "shaking chill" with malaise and fever (temperature, 101 F). The next day he had pain on pressure in the left groin. The following day he had fever (temperature, 102 F.) and tenderness in the right groin. Penicillin was administered at this time. There were no further chills, but the fever and the bilateral inguinal tenderness persisted. A second dose of penicillin was given after 48 hours. His appetite was good, and except for a

slight increase of sweating, no other symptoms were noted. There had been no known contact with domestic cats and no recent scratches of any kind. At the age of 6 years he had had acute glomerulonephritis following scarlet fever. Every August he had had hay fever. The family history was noncontributory.

Physical Examination.—The patient was well nourished and in no acute distress. His skin was free of rash, cyanosis, icterus, and edema. His temperature was 100 F.; pulse rate, 70; respiratory rate, 18. The cervical, epitrochlear and axillary lymph nodes were not enlarged. The inguinal lymph nodes were about the size of prune pits and slightly tender. The heart was not enlarged; there was a Grade 1 apical systolic murmur. The blood pressure was 110/70. There was tenderness in the splenic region, but no masses were palpated.

Laboratory Data.—Urinalysis gave normal results. The hemoglobin content was 14.0 gm.; the red blood cell count, 4,490,000; the leukocyte count 6,900, with polymorphonuclear neutrophiles 49%, lymphocytes 44%, monocytes 2%, and eosinophiles 5%. The erythrocyte sedimentation rate was 16 and 23 mm. per hour (Westergren). A blood culture was negative. Agglutination tests for Brucella abortus, typhoid and paratyphoid A and B Salmonellae, Proteus OX19, and Pasteurella tularensis were negative. Serologic tests were negative; the test for heterophile antibody revealed none. Frei and tuberculin skin tests were negative.

On the day following admission, an inguinal lymph node was excised. One-half was sectioned for microscopic study; the other half was injected into a guinea pig, which was negative for tuberculosis at the end of six weeks.

Pathologic Findings.—The specimen was a hemisegment of an enlarged lymph node, 2.8 cm. in diameter. The external surface was enclosed in a thin connective tissue capsule. The cut surface was studded with single and confluent tan nodules up to 5.0 mm. in diameter, some of which had yellow, purulent centers.

Microscopically, the node was distorted by large pseudotubercles. Each had a central zone of polymorphonuclear neutrophiles and cellular debris, surrounded by a broad zone of epithelioid cells, which, in turn, was surrounded by a collar of lymphocytes. The stroma was crowded with lymphocytes and mononuclear cells.

PATHOLOGY

In gross appearance the enlarged lymph nodes from the four cases described above were strikingly similar. Two specimens consisted of groups of coalescent nodes; the surfaces were lobulated and moderately indurated. The cut surfaces of the four specimens were especially characteristic; they presented a grayish-tan background studded with small yellow tubercles, approximately 3 mm. in diameter; each tubercle had a central core of yellow, purulent material (Fig. 1*A*). The microscopic appearance was distinct in each case. The lymph node structure was partially replaced by discrete pseudotubercles, each revealing a central necrotic zone containing a mixture of fibrin, nuclear debris, and polymorphonuclear neutrophiles (Figs. 1*B* and 2*A*). This zone was surrounded by a collar of epithelioid cells (Fig. 2*B*) which fused with an outer ring of lymphocytes and mononuclear cells. Occasional Langhans-type giant cells were observed, but they were not a constant feature.

The histologic picture observed in these four cases resembles that described in the ulceroglandular form of tularemia.[4] Figure 3 shows a section of an axillary lymph node from a case of tularemia, lent to me by the Armed Forces Institute of Pathology. The patient gave a history of having been tick-bitten five weeks prior to hospitalization. When admitted to the hospital he had acute lymphadenitis involving the right axillary nodes. The agglutination titer for Pasteurella tularensis was 1 to 320.[5]

4. Kavanaugh, C. N.: Tularemia, Arch. Int. Med. **55**:61-85, 1935.

5. De Coursey, E.: Personal communication to the author.

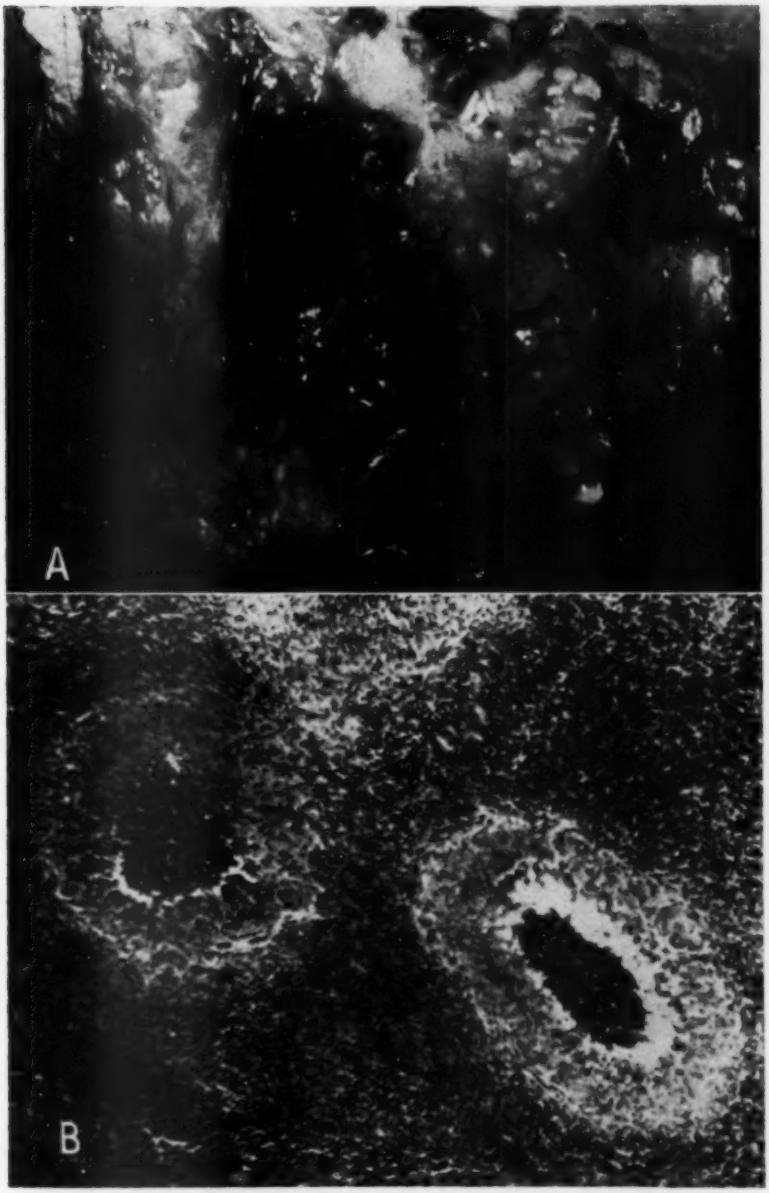

Fig. 1.—*A*, group of enlarged, matted lymph nodes with a necrotic purulent central area. The surface shows scattered tubercles, some of which are oozing pus.

B, low magnification (× 4) of pseudotubercles showing central cores of cellular debris and leukocytes, without caseation.

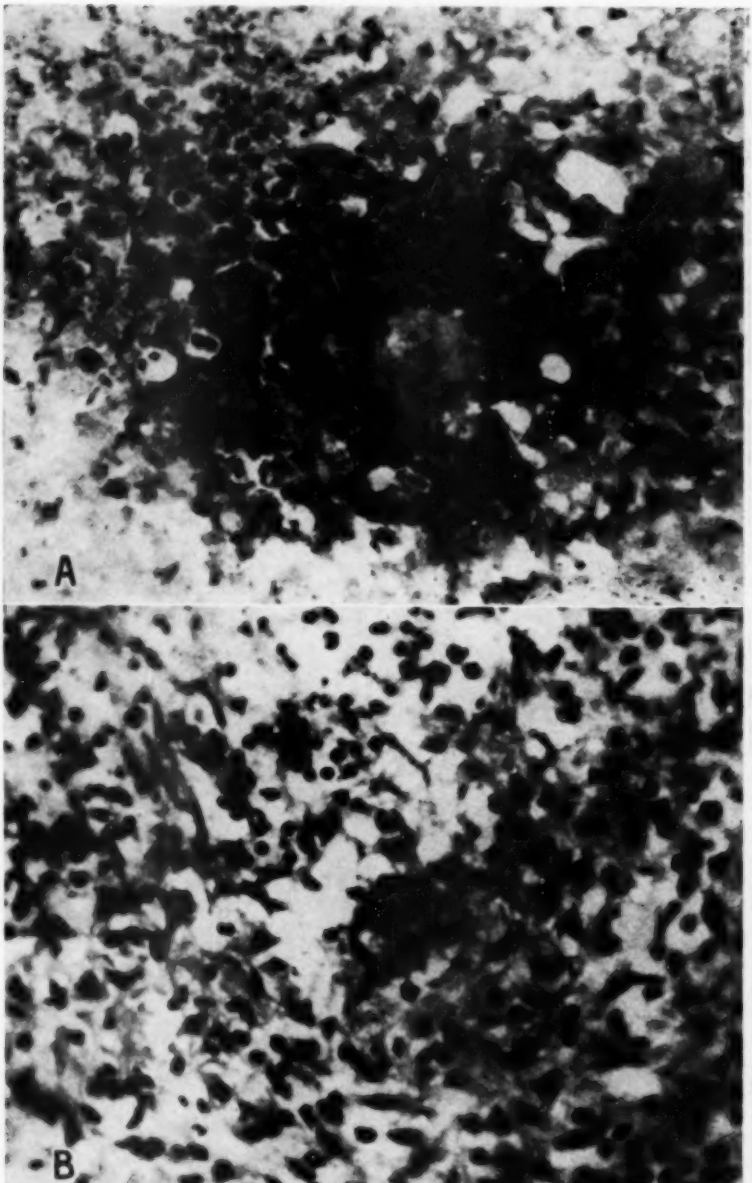

Fig. 2.—*A*, central core of a pseudotubercle, showing cellular debris and leucocytes (× 45). *B*, zone of epithelioid cells surrounding a central core at the right (× 45).

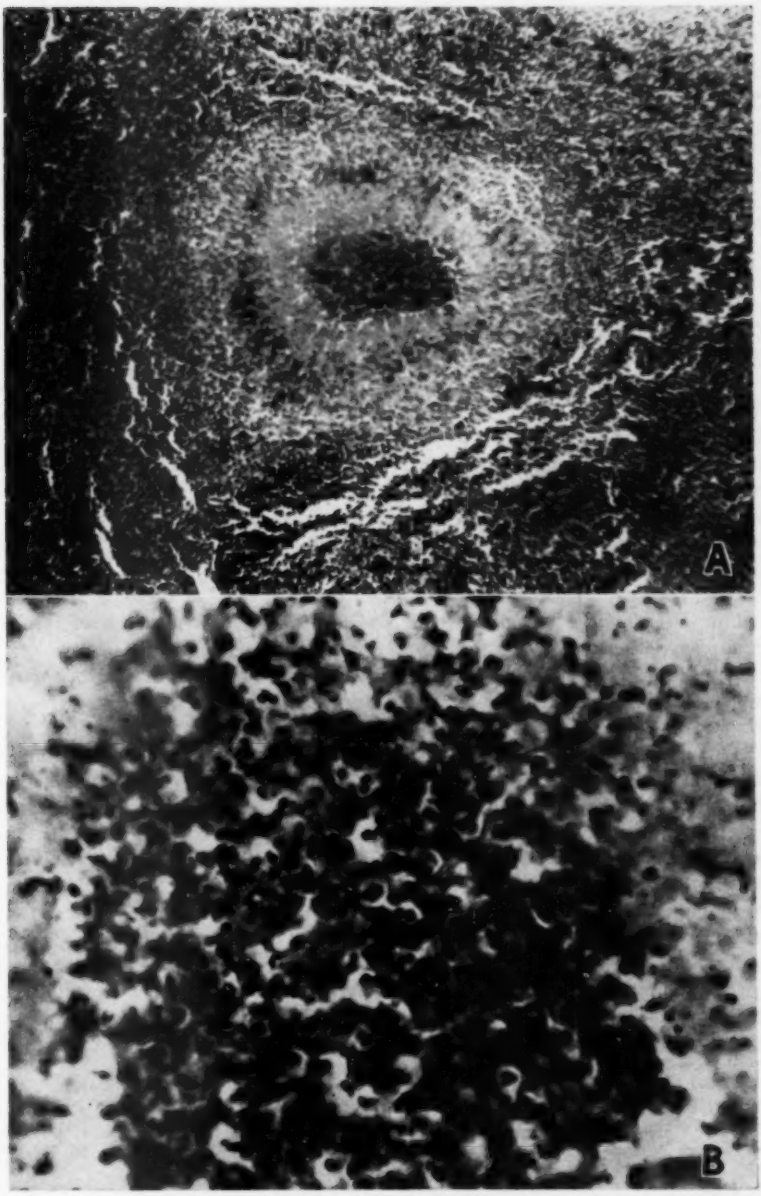

Fig. 3.—*A*, low (× 10) and, *B*, high (× 45) magnification of a pseudotubercle from a case of ulceroglandular tularemia.

COMMENT

In some of the reported cases,[6] organisms of the genus Pasteurella have been isolated from a local lesion following cat-bite or cat scratch, but this has not been a constant result. The organisms found have been classified as Pasteurella multocida or Pasteurella septica, bacteria which are ordinarily not pathogenic for man. However, the isolation of these particular bacteria is of especial interest because the histologic lesion of "cat scratch fever" closely resembles that of tularemia which is caused by Pasteurella tularensis. In none of our cases were agglutination tests for P. tularensis positive. Cultures of portions of lymph nodes excised in two cases were sterile in spite of the fact that specific efforts to support the growth of the Pasteurella group were made. In Cases 2 and 4 the single blood cultures were negative. Thelin and Martin-du-Pan,[1] recording their "New Observations of Disease Due to Cat Scratches," go so far as to say, "The pus aspirated from such nodes is always bacteriologically sterile."

The tendency at present is to presume that the etiologic agent is a virus, probably a member of the psittacosis-ornithosis-venereal lymphomogranuloma group.[7] Experimentally, however, the virus has not been recovered in either test animals or chick embryo culture. A skin antigen prepared from the pus in one case and injected intradermally in other cases has produced a positive skin reaction of the tuberculin type.[8] The patient in the third case above had a positive skin test in 24 and 48 hours with such antigen, which was generously supplied by Dr. Lee Foshay. The antigen, I believe, is from the same stock with which Greer and Keefer [2] obtained a positive reaction in their case. In some of the reported cases,[1] and, in Cases 3 and 4 above, tuberculin and Frei skin tests were negative. Similar results have been reported in the literature.[3] Foshay [7] obtained a low to moderate titer, 1 : 16 to 1 : 128, with antisera from his cases, against lygranum® (chick embryo antigen—an inactivated suspension of the elementary bodies of the virus of venereal lymphogranuloma).

One may speculate about some of the cases previously reported as tularemia on histologic grounds alone, without bacteriologic and/or serologic confirmation. Were they, perhaps, examples of the disease under discussion? A characteristic observation in practically all the reported cases of "cat scratch disease" is that the lymphadenopathy is localized to a single region of the body, most commonly the axilla. In those cases in which there is actual cat scratch, the lymphadenopathy invariably is limited to the regional nodes which drain the "scratch" area. So far as can be determined, the incubation period seems to vary from a week to as long as three months.[8] The disease is apparently not a highly infectious one in that to date cases have been sporadic, and, as a rule, only individual members of a family have been affected. In one case, in which a surgeon was inoculated accidentally at operation, with a needle contaminated by the exudate, no lesion resulted. The dis-

6. (*a*) Allott, E. N.; Cruickshank, R.; Cyrlas-Williams, R.; Glass, V.; Meyer, I. H.; Straker, E. H., and Dee, G.: Infection of Cat-Bite and Dog-Bite Wounds with Pasteurella Septica, J. Path. & Bact. **56**:411-415, 1944. (*b*) Hausmann, G. H., and Tully, M.: Cat-Bite and Scratch Wounds—Consequent Pasteurella Infection of Man, Am. J. Clin. Path. **15**:312-318, 1945.

7. Foshay, L.: Personal communication to the author.

8. Gsell, O.; Forster, R., and Klaus, E.: Virus-Kratz-Lymphadenitis, Schweiz. med. Wchnschr. **81**:699-704, 693-716, 1951.

ease does not appear to be localized to any particular geographic region. Cases have been reported from widely distributed areas of the United States, as well as from Europe. Mollaret and associates [2] claimed it "is more frequent in the country than in cities." The disease is apparently mild and self-limited, although recovery is comparatively slow.

The point to be emphasized is that regional lymphadenopathy, as depicted by the histologic pattern described above, can result from agents other than P. tularensis. Whether these agents are members of the ultramicroscopic virus group or of the Pasteurella group is not definitely established. In none of the case reports of P. septica infection following animal bite or scratch [6a] was a histologic description of enlarged nodes included. Of the six patients mentioned by Allott and associates,[6a] only one had regional lymphadenitis, and histologic examination of the enlarged nodes was not mentioned. To my knowledge, no conclusive evidence has been presented to show that P. septica lymphadenitis is different from any nonspecific suppurative adenitis that accompanies local inflammation. Furthermore, there is no evidence to suggest that it resembles, or is identical with, the histologic lesion described in the four cases above. It should be emphasized again in this connection that Pasteurella organisms were not recovered in two of the cases described above in which bacteriologic study was made.

SUMMARY

Four cases of presumptive "cat scratch fever" are presented, only two of which were associated with "cat scratch."

In histologic appearance the involved lymph nodes examined in the four cases were strikingly similar. The histologic appearance cannot be distinguished from that of tularemia.

Agglutination tests against Pasteurella tularensis were negative in all four cases.

The disease is only slightly incapacitating and is self-limited without specific therapy.

The etiologic agent and the mode of transmission have not been established.

Although domestic cats are associated with a high percentage of cases, the disease occurs in the absence of known contact with cats.

Dr. John D. Booth of Danbury, Conn., Dr. Maxwell Eddy of Bridgeport, Conn., Dr. Eugene Bogardus of Pleasantville, N. Y., and Dr. F. Morgan Pruyn of Mount Kisco, N. Y., gave me permission to use their cases.

The photographs were supplied by Mr. John Orr.

CARDIOVASCULAR LESIONS AS A RESULT OF JOINING RATS IN PARABIOSIS

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IN SPITE of extensive work done on parabiotic rats by many investigators, it is only recently that a satisfactory explanation has been given for the difficulties commonly encountered in maintaining the animals in good condition. Hall and Hall [1] found that when rats were joined in parabiosis and survived for at least four weeks, in 8 out of 20 pairs hypertension developed in one of the partners, associated with extensive vascular lesions of the heart, the kidneys and the mesentery, consisting of hypertrophy, sclerosis, hyalinization, and perivascular infiltrations of inflammatory cells.

Finerty and Panos,[2] however, in their experiments on "parabiotic intoxication," made no statement about arterial lesions in pairs of rats in which one rat presented intense hyperemia and the other rat became cachectic and died. They reported that autopsy revealed the larger surviving partner to be essentially normal, while the smaller rat showed adrenal hypertrophy and thymus atrophy. The onset of intoxication occurred before the 21st postoperative day. They reviewed the literature, indicating the different points of view in regard to "parabiotic disharmony."

Other results were obtained by Van Dyke and Huff,[3] who stated that with a pure strain of rats (brother-sister matings for 50 generations) no incompatibilities developed during parabiosis. In my previous experiments, litter-mates of a strain of Wistar albino rats bred in this laboratory for many years were used. Although various difficulties that led to death of the rats were at times encountered, in many instances the animals survived during long-time experiments.

In the present experiments, which were begun for the purpose of transplanting tumors to one parabiotic partner (a procedure not relevant to the observations reported in this paper), the rats used were young male Wistar albinos that were not of a closely inbred strain. It was thought of interest to find out whether vascular lesions occurred during these short-time experiments.

Hall and Hall made their study chiefly upon rats that survived four weeks or longer, and so were concerned with late lesions. The present series includes lesions of duration shorter than four weeks; that is, early lesions. The results indicate an astonishingly early development of necrotic arteritis.

From the Department of Pathology, University of Pennsylvania School of Medicine.

1. Hall, C. E., and Hall, O.: Production and Pathogenesis of Parabiotic Hypertension in the Rat, A. M. A. Arch. Path. **51**:527, 1951.

2. Finerty, J. C., and Panos, T. C.: Parabiosis Intoxication, Proc. Soc. Exper. Biol. & Med. **76**:833, 1951.

3. Van Dyke, D. C., and Huff, R. L.: Epilation in the Non-Irradiated Member of Parabiotically United Rats, Proc. Soc. Exper. Biol. & Med. **72**:266, 1949.

Experiments Are Arranged in Order of Time Interval After Parabiotic Operation

			Partner A							Partner B			
				Lesions in Heart									
Exper. No. (Pairs)	Litter-Mates	Days After Parabiosis	Mode of Death	Inflam. Cells Surrounding Vessels	Subendothelial Necrosis of Vessel Walls	Fibroblastic Proliferation Around Vessels and Replacing Injured Myocardium	Kidney Lesions; Thickening of a Few Small Arteries	Inflam. Cells in Walls and Around Mesenteric Vessels	Adrenal Lesions	Mode of Death	Lesions in Coronary Vessels	Kidney Lesions	Adrenal Lesions
55	Yes	16	Fd. dead	0	0	0	—	—	Congestion	Cachexia; fd. dead	0	0	0
50	Yes	18	Fd. dead	0	0	0	—	—	0	Cachexia; fd. dead	—	—	—
33	Yes	16	Killed	+++	—	+++	0	0	0	Cachexia; fd. dead	—	—	—
31	Yes	18	Killed	+++	++++	++++	+	0	0	Cachexia; fd. dead	0	0	Congestion
58	Yes	20	Killed	0	0	0	0	—	—	Cachexia; killed	—	—	—
18	No	20	Killed	++	0	++	0	0	—	Cachexia; fd. dead	0	0	Congestion
21	No	20	Killed	+	++++	++++	+	++	Hemorrhage	Fd. dead	0	0	—
1	No	22	Killed	++	++++	++++	0	0	—	Cachexia; fd. dead	0	0	Congestion
25	Yes	25	Killed	++	0	++	+	0	0	Cachexia; killed	0	0	0
17	No	26	Cachexia; fd. dead	++	+	+++	0	—	—	Cachexia; fd. dead	0	0	—
7	No	29	Fd. dead	0	0	0	0	0	—	Small rat killed	0	0	0
47	Yes	29	Killed	+	—	+	—	—	—	Smaller rat killed	—	—	—
4	No	31	Killed	+	0	+	0	—	—	Smaller rat killed	0	0	0
36	Yes	32	Killed	+++	—	+++	0	+++	Congestion	Cachexia; killed	0	0	0
24	No	35	Killed	+	0	++	0	—	—	Cachexia; killed	0	0	—
3	No	45	Killed	+	0	+++	+	++++	0	Cachexia; fd. dead	—	—	—

0 = no lesion in microscopic section studied.
— = tissue not available for sectioning.

MATERIAL AND METHODS

The numbers of rats joined in parabiosis were 26 litter-mate pairs and 28 pairs that were not litter-mates, totaling 54 pairs, but only 16 pairs were used for histologic study. All were young males, weighing between 100 and 150 gm., and not of an inbred colony. Six litter-mate pairs died, and four pairs were killed, in all of which one partner was smaller. Tissues of 8 of these 10 pairs were examined microscopically. Twelve non-litter-mate pairs died, and two pairs were killed, in all of which one partner was smaller. Of these 14 pairs, 8 were studied histologically. The number of days that elapsed after joining the rats in parabiosis is given in the accompanying table. Sometimes the tissues of the cachectic rats that were found dead were too autolyzed to permit histological study.

RESULTS

In all the 16 pairs of rats studied histologically, there had developed a disparity in growth between the partners. One member of each pair had failed to grow normally, often became weak, pale, and cachectic, and usually died. This rat (designated in the table as Partner B) never showed any vascular lesions, and no important lesions were found at autopsy except congestion of the internal organs. The other rat of the pair (designated in the table as Partner A) was typically a vigorous, healthy-appearing animal, but on the death of its partner was found, in many instances, to have extensive lesions in and around blood vessels. Sometimes this larger rat (Partner A) had passed through a stage of extremely active hyperemia, such as was described by Finerty and Panos.[2]

Figures 1 to 4 illustrate lesions observed in the five Partner A rats that were found to have the most severe changes in blood vessels. The earliest lesions of the coronary arteries were marked by perivascular collections of inflammatory cells (Fig. 1*A* and *B*). The predominant cells were histiocytes and lymphocytes, with a scanty admixture of polymorphonuclear leukocytes. Proliferation of fibroblasts occurred early (Figs. 2*A* and 3*A*). In three rats necrotic and hyalinized areas were found under the endothelium (Figs. 1*A* and *B*, 2*A*, and 3*A*), staining heavily with eosin. The arterial involvement was extensive; often a low-power microscopic field would include three damaged vessels. A wide cuff of inflammatory cells surrounded these vessels. Myocardial muscle fibers had disintegrated in the neighborhood, and fibroblasts proliferated extensively in replacing the injured areas (Figs. 1*B* and *C*, 2*A*, and 3*A*). Some muscle fibers were hyalinized (Figs. 2*B* and 3*B*). At later intervals, dense scar tissue had formed (Fig. 3*B*). The most severe necrotic changes were observed at 18, 20, and 23 days after operation. The hyalinized necrotic areas in the wall and the swelling of the endothelium caused severe narrowing of the lumen. Lesions occurred in the arteries of both ventricles in some rats.

Kidney lesions were slight or absent. Only in four rats were there occasional thickened small arteries, and these were sclerotic rather than inflammatory lesions. Gross hemorrhages, scars, and contractions were not observed, but occasional glomeruli and tubules were atrophied. No significant lesions were found in the adrenals, except for hemorrhage in one rat that had severe vascular lesions in the heart. One rat with severe arterial lesions had a superficial hemorrhagic ulcer of the stomach.

Inflammatory lesions were found in the mesenteric vessels in three rats, with histiocytes, polymorphonuclear leukocytes, eosinophiles, and lymphocytes infiltrating the walls of the vessels and surrounding them. The most severe changes (Fig. 4)

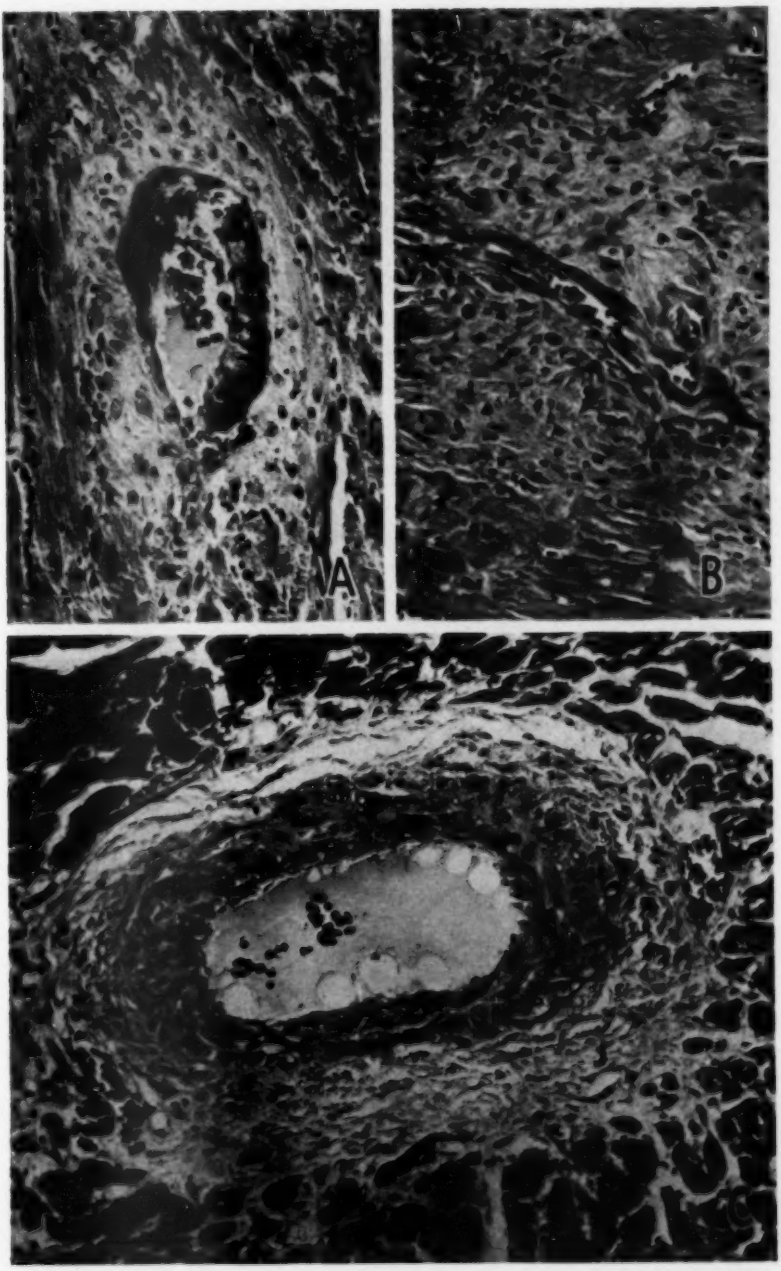

Fig. 1.—*A*, Pair 1, 23 days after parabiosis. Patches of hyaline necrosis occur under the endothelium of a coronary vessel. Perivascular mononuclear phagocytes form a cuff around the vessel. × 270.

B, Pair 21, 18 days after parabiosis. Subendothelial hyaline necrosis stains deeply. Fibroblastic proliferation replaces destroyed myocardium. × 270.

C, Pair 3, 45 days after parabiosis. A late stage of perivascular fibroblastic proliferation is shown. × 270.

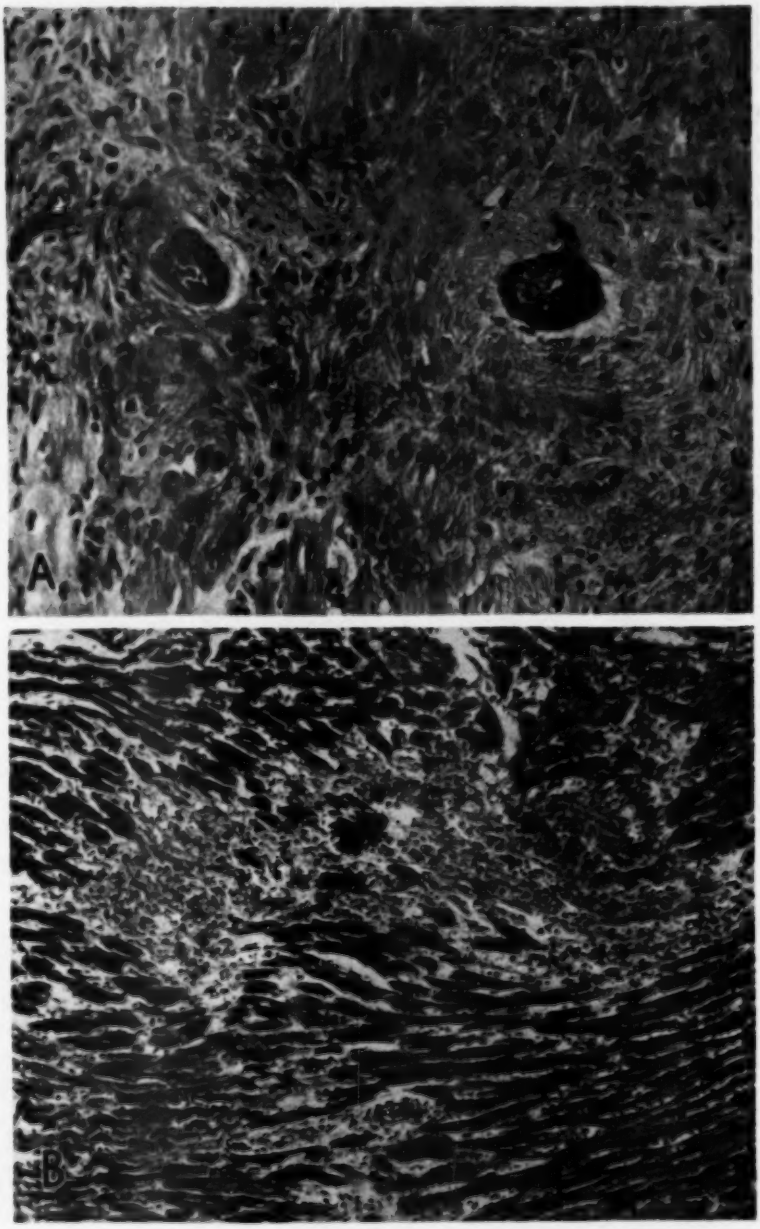

Fig. 2.—*A*, Pair 31, 18 days after parabiosis. Intense staining of subendothelial hyaline necrosis is seen, with perivascular mononuclear infiltration and fibroblastic proliferation, replacing destroyed heart muscle cells. The lumen of each vessel is greatly narrowed by the swollen necrotic vessel wall. × 240.

B, Pair 33, 18 days after parabiosis. Intense staining of injured myocardial muscle is seen. Areas of inflammatory cells and fibroblastic proliferation occur where vascular occlusions have caused areas of necrosis in the myocardium. × 125.

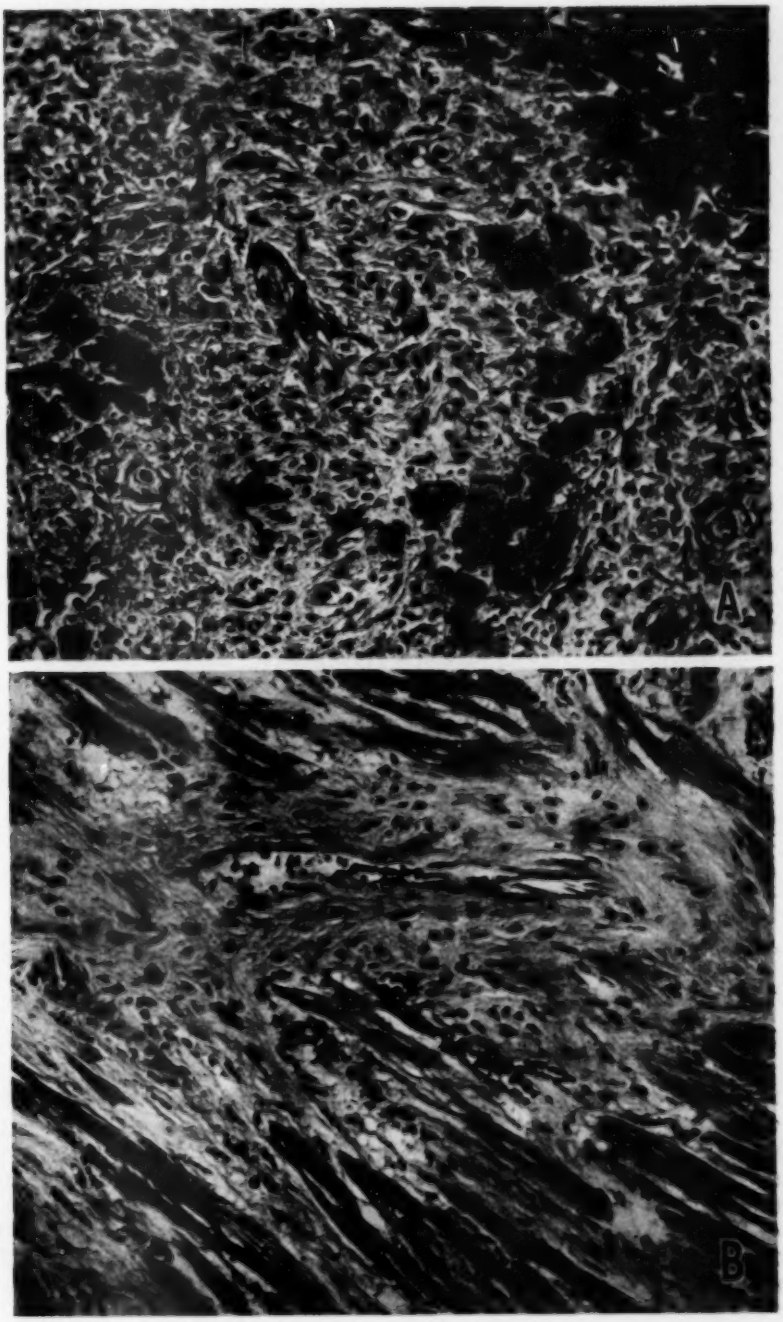

Fig. 3.—*A*, Pair 1, 23 days after parabiosis. A little to the left of the center, and also in the right lower corner, are necrotic arterioles surrounded by fibroblastic proliferation. Surviving myocardial fibers are scattered between the two areas. Masson stain; × 270.

B, Pair 3, 45 days after parabiosis. A late scarred area in the myocardium. × 270.

were seen in the longest interval studied, 45 days after parabiosis, in a rat which had healing lesions in the heart (Fig. 1*C*). The mesenteric vessels appeared grossly as thick, nodular cords, typical of periarteritis nodosa, and microscopically many of these vessels showed subendothelial patches of intensely eosinophilic hyaline material replacing destroyed cells of the wall (Fig. 4). External to this there was a wide cuff of inflammatory cells and fibroblasts. In some vessels, endothelium had been destroyed, and thrombosis had occurred.

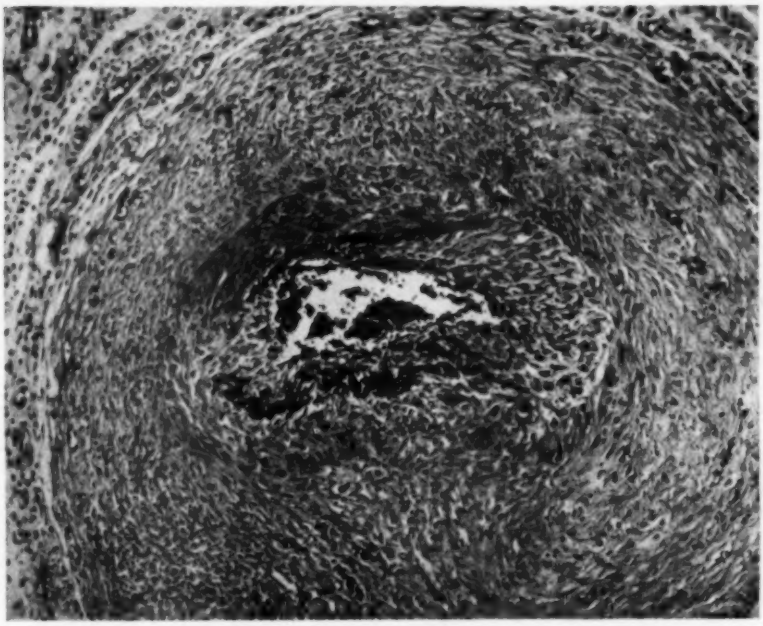

Fig. 4.—Pair 3. One of many similar lesions observed in branches of mesenteric arteries of a rat 45 days after parabiosis. Hyalinization and necrosis are seen in irregular patches that stained intensely with eosin and appear in the photograph as black lines in the intima. Inflammatory cells infiltrate the entire wall and surrounding tissues.

COMMENT

Hall and Hall in their first paper [1] dealt with lesions developing 29 to 45 days postoperatively, although they stated that the most advanced cardiovascular lesions could develop as early as 21 days postoperatively. They interpreted the lesions as consistent with malignant hypertensive cardiovascular renal disease, periarteritis nodosa, and rheumatic myocarditis. They suggested three hypotheses: (1) Parabiosis elicits an alarm reaction; (2) one rat causes an allergic reaction in its partner; (3) one rat's serum contains specific substances toxic to the kidneys of the partner. They stressed the point that the cardiovascular lesions are associated with the hypertensive state, that no lesions occur in normotensive rats.

The probability that salt played a role, since their first series of rats had received 1% NaCl solutions,[1] led Hall and Hall to make a subsequent study [4] of the effect of giving parabiotic rats tap water (which naturally contained 31.5 to 41.5 mg. of sodium per 100 ml.) and distilled water. They found that in the rats given tap water the same vascular lesions developed as in those given 1% sodium chloride solution, except that the former did not have edema. Those given distilled water were not found to have lesions in arteries of the pancreas and the mesentery but had microscopic lesions in arteries of the heart and the kidneys. In their experiments some 150 litter-mate female rats were united in parabiosis. Rats showing parabiotic intoxication and those in poor health or with impaired growth were discarded. From the data one judges that about 50% of their rats were discarded. Of 19 pairs given tap water, hypertension developed in one partner of 8 pairs, and 5 of these showed arterial lesions. Of 19 pairs given distilled water, hypertension developed in 7, and 4 of these had vascular lesions in the heart and the kidneys of one of the partners. Time intervals are not given, but it is implied that the rats were killed after a number of weeks.

In my series the rats were of the type that Hall and Hall discarded. Hall and Hall dealt with lesions compatible with apparent good health of both partners, in spite of the hypertension of one partner. In my series I deal mainly with the early lesions of one parabiont whose partner has a cachexia incompatible with life and whose tissues presumably are causing a severe toxic effect in the partner.

Since the arterial lesions in heart and mesentery are distinctly inflammatory in character, one must consider the possibility of allergic reaction. The lesions appear to be very similar to those produced in rabbits by sensitizing them to foreign serum (Rich and Gregory [5]). It would seem possible that the anemic, cachectic rat was releasing substances that might act as antigens injected into its parabiont. Against this is the fact that in the rat it is difficult to produce a manifestation of allergy unless the adrenals are removed. The intense hyperemia of some rats that later were found to have arterial lesions may be similar to the flushing of the ears and increased body temperature occurring in rabbits one week after injection of foreign serum.[5]

In another paper, Hall and Hall [6] inclined to the view that the pathological changes may be due to adrenal mineralocorticoid response to conditions incident to parabiosis. The inflammatory lesions of vessels were similar to those resulting from massive doses of desoxycorticosterone acetate and of crude anterior pituitary extracts injected in unilaterally nephrectomized animals that were given sodium chloride drinking fluid (Selye [7]).

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4. Hall, C. E., and Hall, O.: The Relationship of Sodium Intake to the Hypertensive Hyalinosis Syndrome Produced in the Rat by Parabiosis: I. Hypertensive Cardiovascular Disease, Texas Rep. Biol. & Med. **9**:714, 1951.

5. Rich, A. R., and Gregory, J. E.: Experimental Demonstration That Periarteritis Nodosa Is Manifestation of Hypersensitivity, Bull. Johns Hopkins Hosp. **72**:65, 1943.

6. Hall, O., and Hall, C. E.: The Relationship of Sodium Intake to the Hypertensive Hyalinosis Syndrome Produced in the Rat by Parabiosis: II. Arthritis, Texas Rep. Biol. & Med. **9**:728, 1951.

7. Selye, H.: General Adaptation Syndrome and Diseases of Adaptation, J. Clin. Endocrinol. **6**:117, 1946.

In my series there was absence of any constant changes in adrenals, except the changes in size and congestion that may be associated with any operative incision. There seems to be no proof that endogenous hormones secreted by the adrenals are etiological factors.

The vascular lesions here described seem exactly similar to those recently reported by Masson,[8] who produced them by three different methods: (1) wrapping the kidneys in silk to produce hypertension, (2) injecting large doses of desoxycorticosterone acetate in unilaterally nephrectomized rats given sodium chloride, (3) injecting anterior pituitary extracts in unilaterally nephrectomized rats. A common factor of all three methods was vascular hypertension, which Masson considered the most significant pathogenic factor. Previous descriptions of similar arterial lesions of rats have been recorded after experimental production of hypertension by constricting the kidney.[9]

If hypertension is the chief etiological factor in the production of these arteriolar lesions, the question remains: How can a parabiotic operation in itself cause hypertension, as demonstrated by Hall and Hall? Although they describe arteriolar sclerosis of the kidneys, they speak of the kidneys as being hypertrophied. In that case there would seem to be no ischemic, contracted kidney of the type known to produce substances that initiate hypertension. In my series it seems evident that kidney lesions are negligible, not of primary importance.

Lesions apparently histologically identical have been reported to occur in alloxan-treated rats given sodium chloride by Chute and co-workers,[10] who stated that the "vascular lesions are produced by the action of sodium chloride in those cases in which the kidneys have been damaged by alloxan." Their data concerning terminal blood pressure readings on these rats indicate that vascular lesions were found in some rats that were not hypertensive. They found the vascular lesions most pronounced in the kidney.

Wilens and Sproul[11] have described as periarteritis nodosa lesions in 9.7% of 487 rats dying of natural causes. These lesions never appeared before the 500th day of life, only occasionally before the 700th day. They occurred in coronary, mesenteric, renal, and other arteries. None of the rats in my series was over 4 months of age.

SUMMARY

Sixteen pairs of young albino Wistar rats (not a pure strain) that had been joined in parabiosis and had shown signs of "parabiotic disharmony" were studied

8. Masson, G. M. C.; Hazard, J. B.; Corcoran, A. C., and Page, I. H.: Experimental Vascular Disease Due to Desoxycorticosterone and Anterior Pituitary Factors: Comparison of Pathologic Changes, Arch. Path. **49**:641, 1950.

9. Smith, C. C.; Zeek, P. M., and McGuire, J.: Periarteritis in Experimental Hypertensive Rats and Dogs, Am. J. Path. **20**:721, 1944. Smith, C. C., and Zeek, P. M.: Role of Various Factors in Etiology of Periarteritis Nodosa in Experimental Animals, ibid. **23**:147, 1947. Zeek, P. M.; Smith, C. C., and Weeter, J. C.: Studies on Periarteritis Nodosa: III. Differentiation Between Vascular Lesions of Periarteritis Nodosa and Hypersensitivity, ibid. **24**:889, 1948.

10. Chute, A. L.; Orr, J. L.; O'Brien, M. J., and Jones, E. E.: Vascular Lesions in Alloxan Diabetic Rats, A. M. A. Arch. Path. **52**:105, 1951.

11. Wilens, S. L., and Sproul, E. E.: Spontaneous Cardiovascular Diseases in the Rat: II. Lesions of the Heart, Am. J. Path. **14**:201, 1938.

histologically at periods of 16 to 45 days postoperatively. In each of the 16 pairs one partner failed to grow normally, was smaller, usually became cachectic, and died. The other partner appeared as a vigorous, healthy animal, sometimes passed through a stage of hyperemia, and in 12 cases had at autopsy inflammatory and sometimes necrotic lesions in the coronary arteries of the right and left ventricles. Extensive lesions of the coronary arteries occurred within 18 days after parabiotic union.

The arterial lesions in the heart, and the less frequent lesions in the mesenteric vessels, were similar to those first described by Hall and Hall as resulting from parabiotic operation alone. In contrast to their findings, however, in my series renal lesions were absent or negligible.

It is of interest that the vascular changes invariably appeared in the rat that was apparently healthy and not in the cachectic partner, which showed no damage except congestion.

The pathogenesis of the vascular changes cannot as yet be explained.

POLYSACCHARIDE NATURE OF CORPORA AMYLACEA

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THE TERM corpora amylacea is used to denote the laminated intraglandular bodies of the prostate which were first described in 1723 by Morgagni.[1] Virchow noted that they were iodophilic, and today these two criteria, the laminated structure and the iodophilic staining reaction, are stated to be the distinguishing features of corpora amylacea. The term is also applied to certain bodies in the lung, the brain, the spinal cord, and tooth pulp.

In the prostate the corpora are round or faceted by the pressure of adjacent corpora and are composed of concentric laminae about a central nidus. They occur in the gland lumina, alone or in numbers, are commoner in senility, and vary in size up to several hundred microns in diameter. From an extensive study of their morphology, Moore,[2] in 1936, concluded that they were formed from desquamated epithelial cells and prostatic secretion and that cyclic growth occurred by the addition of concentric layers and lines. Those in the lung are similar in appearance to those in the prostate, but they occur singly in the alveoli and so are round. They vary in size as do those in the prostate.

In the nervous system corpora amylacea appear as discrete spherical, usually laminated, extracellular bodies varying from 15 to 50 μ in diameter. They are most commonly found in the subependymal, subpial, and perivascular regions of the brain and spinal cord. Their numbers are increased in old age and in relation to degenerative foci, thus suggesting that they represent reactive changes to degeneration. It has been proposed that they arise from the products of tissue destruction [3] or, more

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1. Weil, A.: Textbook of Neuropathology, Ed. 2, New York, Grune & Stratton, Inc., 1945, p. 11.

2. Moore, R. A.: Morphology of Prostatic Corpora Amylacea and Calculi, Arch. Path. 22:24, 1936.

3. Greene, T. H.: Manual of Pathology, revised by H. W. C. Vines, Ed. 17, Baltimore, Williams & Wilkins Company, 1949, p. 1073.

specifically, from degenerating axis cylinders,[4] that they are altered forms of oligodendroglia or fibrillary astrocytes,[5] or are degenerated microglia.[6]

The chemical nature of the corpora amylacea is not known. Some factors suggest variations in composition in different locations, such as the fact that in the lung and the prostate they are eosin-positive and in the nervous system and the tooth pulp they are hematoxylin-positive. Those of the prostrate are most accessible for study, and certain conclusions have been made concerning their nature. Their iodophilic property suggests the presence of starch or glycogen. Moore and Hanzel,[7] in 1936, concluded that those in the prostate were composed largely of protein and nucleic acid, probably in the form of nucleoprotein, and while small amounts of lipids and amyloid were also found, they were not considered important constituents. None of their tests for carbohydrate was positive. Less is known concerning those in the nervous system. It has been stated that they contain neither amyloid nor lipids.

Certain stains have recently become available for the cytochemical study of tissue, and while most tissues have been investigated in this manner, no reports are available on the corpora amylacea.[8] Thus it was of interest to examine these bodies from the prostate, the lung, and the brain by the new methods.

MATERIAL AND METHODS

The tissues containing corpora amylacea which were examined cytochemically were derived partly from fresh surgical material and partly from postmortem specimens. All tissues were fixed in 10% formalin and cut, after paraffin embedding, at thicknesses necessary for the investigation. The desoxyribosenucleic acid was estimated by means of the Feulgen reaction as previously described (Leuchtenberger,[9] 1950). Azure A was used for the testing of ribosenucleic acid according to Flax and Pollister[10] (1949). This method was combined with the use of a purified crystalline ribonuclease. For the determination of the polysaccharides, the periodic acid-Schiff technique as modified by Hotchkiss[11] was employed in combination with the use of hyaluronidases, amylases, and other enzymes as discussed by Leuchtenberger and Schrader[12]

4. Saxen, A.: Über die Genese der Corpora amylacea im Zentralnervensystem, Virchows Arch. Path. Anat. **300**:534, 1937.

5. Courville, C. B.: Pathology of the Central Nervous System: A Study of Lesions Found in a Series of 30,000 Autopsies, Ed. 2, Mountain View, Calif., Pacific Press Publishers Association, 1945, p. 42.

6. Boyd, W.: Textbook of Pathology: Introduction to Medicine, Ed. 5, Philadelphia, Lea & Febiger, 1945, p. 868.

7. Moore, R. A., and Hanzel, R. F.: Chemical Composition of Prostatic Corpora Amylacea and Calculi, Arch. Path. **22**:41, 1936.

8. Reports have been published of periodic acid-Schiff-positive tissues, some of which have included corpora amylacea (Leblond, C. P.: Distribution of Periodic Acid-Reactive Carbohydrates in the Adult Rat, Am. J. Anat. **86**:1, 1950. Lillie, R. D.: Histochemical Comparison of the Casella, Bauer and Periodic-Acid Oxidation-Schiff Leucofuchsin Technics, Stain Technol. **26**:123, 1951.)

9. Leuchtenberger, C.: A Cytochemical Study of Pycnotic Nuclear Degeneration, Chromosoma **6**:449, 1950.

10. Flax, M., and Pollister, A. W.: Staining of Nucleic Acids by Azure A, Anat. Rec. **105**:536, 1949.

11. Hotchkiss, R. S.: A Microchemical Reaction Resulting in Staining of Polysaccharide Structures in Fixed Tissue Preparations, Arch. Biochem. **16**:131, 1948.

12. Leuchtenberger, C., and Schrader, F.: The Chemical Nature of the Acrosome in Male Germ Cells, Proc. Nat. Acad. Sc. **36**:677, 1950.

(1950). The testing for basic amino acids was done by means of fast green staining as used by Schrader and Leuchtenberger [13] (1950). The results are summarized in Tables 1 and 2.

COMMENT

On the basis of the cytochemical findings, it appears that the corpora amylacea of the prostate, the lung, and the brain do contain a polysaccharide with a 1,2 glycol grouping. According to McManus and Cason,[14] this is demonstrated by blocking these groups with acetic anhydride and unblocking them with potassium hydroxide, which produce a negative and a positive periodic acid-Schiff reaction, respectively. Glycogen, starch, hyaluronidase, and glycolipids were not found to be present and so are not considered responsible for the positive periodic acid-Schiff reaction.

Although the corpora of all three tissues give a positive periodic acid-Schiff reaction, the intensity of the reaction varies considerably from corpus to corpus in lung

TABLE 1.—*Polysaccharides Demonstrated in Corpora Amylacea of Different Tissues by Means of the Periodic Acid-Schiff Reaction (PAS Reaction)*

Organ	PAS	PAS After Acetic Anhydride	PAS After Acetic Anhydride and KOH	PAS After Hot Methanol Chloroform	PAS After Saliva or Amylase	PAS After Hyaluronidase
Prostate	+	−	+	+	+	+
Lung	+	−	+	+	+	+
Brain	+	−	+	+	+	+

TABLE 2.—*Comparison of Various Specific Staining Reactions of Corpora Amylacea of Different Tissues*

Organ	Feulgen for Desoxyribosenucleic Acid	Azure A with Ribonuclease for Ribosenucleic Acid	Fast Green for Basic Groups of Proteins
Prostate	−	−	+
Lung	−	−	+
Brain	−	−	−

and prostate, suggesting that in these organs, at least, there is a variation in the concentration of the common constituent. This variation was not apparent in the corpora of the brain. Also to be noted in the sections of prostate and lung is that while the periodic acid-Schiff reaction is positive following acetylation and treatment with potassium hydroxide, it is not so strongly positive as in the sections not acetylated. Thus some of the positive material has been altered or lost in the acetylation reactions.

Since it has been reported that the corpora amylacea of the prostate contain nucleoprotein, the corpora of prostate, lung and brain were tested for the presence of desoxyribosenucleic acid and ribosenucleic acid. As is shown in Table 2, neither of these acids was found to be present. Those of the lung and the prostate did show

13. Schrader, F., and Leuchtenberger, C.: A Cytochemical Analysis of the Functional Relations of Various Cell Structures in Arvelius Albopunctatus (De Geer), Exper. Cell Res. **1**:421, 1950.

14. McManus, J. F. A., and Cason, J. E.: Carbohydrate Histochemistry Studied by Acetylation Techniques: I. Periodic Acid Methods, J. Exper. Med. **91**:651, 1950.

the presence of a basic protein. The absence of the nucleoprotein and the presence of the polysaccharide do not confirm the work of Moore and Hanzel,[7] who found by chemical analysis that nucleoprotein was present and carbohydrate absent.

The corpora of the prostate are generally considered to be formed from desquamated epithelial cells and prostatic secretion. So far as we can determine, there is no direct evidence in the literature that cells are necessary for the formation of corpora amylacea. The lack of desoxyribosenucleic and ribosenucleic acid suggests that the corpora may have originated from glandular secretion without the addition of cells or cellular constituents. In the presence of stagnation, due either to obstruction or to the decrease of secretion of old age, it is suggested that inspissation of secretion could occur with the production of what we call corpora amylacea. Those of the lung may be similarly formed from inspissated bronchial secretion. Very little can be postulated about those in the brain, other than that the lack of nucleic acid does not support the theories of corpora amylacea formation based on a cellular origin.

The alternative is, of course, that cells do enter into the formation of corpora amylacea but that the nucleic acid is altered or lost so that we have found no evidence of its presence. This is conjecture and should not detract from the fact that nucleic acid is not present in the corpora.

CONCLUSIONS

1. Corpora amylacea of the prostate, the lung, and the brain contain a polysaccharide with a 1,2 glycol grouping.

2. The concentration of the polysaccharide varies in individual corpora in the prostate and the lung.

3. Nucleic acid, either as desoxyribosenucleic or ribosenucleic acid, is not present.

4. It is suggested that the corpora amylacea of the prostate and the lung are formed from inspissated secretion and that there is no evidence of cells as a constituent.

Dr. J. Lowell Orbison gave assistance in this study.

A COMPARATIVE STUDY OF THE REACTION TO INJURY

I. The Cellular Response to Methylcholanthrene and to Talc in the Body Cavity of the Cockroach (Periplaneta americana)

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THE STUDY of the reaction to injury of invertebrates and lower vertebrates has been largely concerned with an investigation of the replacement of lost parts,[1] the healing of surface wounds,[2] and the role played by phagocytes in defense against invading micro-organisms.[3] Relatively little work has been carried out on the aspects of the reaction to injury which in man would be identified as chronic inflammation, with its associated reactive hyperplasia, metaplasia, and repair. These will be the subject of the present study.

One facet of this inquiry has been given particular attention by Russian investigators, notably Maximow [4] and Zawarzin and his students,[5] who sought to clarify the relationship between various blood and connective tissue elements by studying the inflammatory response in vertebrate, as well as in invertebrate, animals. Also, parsitologists, in their examination of the interaction between host and parasite, have made numerous contributions to the comparative study of the reaction to injury.

The choice of an insect with which to begin this series was determined by the realization that, from the standpoint of number of species and of individuals, insects today are the dominant animals on earth. The total number of known animal species is about 850,000; of these, 625,000 are insects. The American cockroach (Periplaneta americana) was employed for several reasons: It is large and hardy, has an adult life span of a year or more, and has been the subject of extensive research. The cockroach retains many characteristics of the primitive, less specialized insects.[6]

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1. Korschelt, E.: Regeneration und Transplantation, Berlin, Gebrüder Borntträger, 1927-1931.

2. (*a*) Arey, L. B.: Wound Healing, Physiol. Rev. **16**:327-406, 1936. (*b*) Wigglesworth, V. B.: Wound Healing in an Insect (Rhodnius Prolixus), J. Exper. Biol. **14**:364-381, 1937.

3. Metchnikoff, E.: Lectures on the Comparative Pathology of Inflammation, translated by F. A. Starling and C. H. Starling, London, 1893.

4. Maximow, A. A.: The Relation of Blood Cells to Connective Tissue and Endothelium, Physiol. Rev. **4**:535-563, 1924.

5. Zawarzin, A.: Beiträge zur vergleichende Histologie des Blutes und Bindegewebes: I. Allgemeine Einführung, Ztschr. mikr. Anat. Forsch. **3**:367-373, 1925.

6. Miall, L. C., and Denny, A.: The Structure and Life History of the Cockroach, London, L. Reeve & Co., 1886.

Roaches reached their greatest expansion during the Upper Carboniferous Age, when they comprised 60% of the insectan fauna, compared with 1% today.[7] It is intriguing to speculate whether in these studies we are witnessing a mechanism of response to injury that has remained essentially unchanged for a quarter of a billion years!

MATERIALS AND METHODS

Sexually mature male and female roaches were used in these experiments. Reproduction occurred without hindrance among animals previously operated upon; the young nymphs showed no abnormalities and were destroyed. The insects were kept in cans with perforated covers and were constantly supplied with cubes of dog chow[8] and water.

Methylcholanthrene and talc (magnesium silicate) in powdered form, moistened with 0.85% saline, were the irritants used. The former was chosen because it is known to be carcinogenic in some vertebrate animals and because it can be identified in tissues by its fluorescence under ultraviolet rays and in sections by the shape of the crystals and crystal spaces. Talc produces a granulomatous inflammation in vertebrates and can readily be identified in tissue sections under polarized light by its property of double refraction.

Each insect was anesthetized before operation by dropping it into a narrow jar filled with carbon dioxide gas delivered through a short section of rubber tubing from a bottle containing several pieces of solid carbon dioxide. Within 15 to 30 seconds the roach was quiet and could be transferred to a cork board, where the wings were clipped. A 1- to 2-mm. transverse incision was then made in the dorsal or the ventral surface between the third and fourth abdominal segments. The incision was located lateral to the midline to avoid injuring the dorsal tubular heart or the ventral nerve cord.

Small pellets of methylcholanthrene or of talc were gathered on the end of a probe and pressed through the slit-like incision into the body cavity. The entire procedure was usually accompanied by the loss of not more than a drop of hemolymph and was completed in two to three minutes. At the end of this time the effects of the anesthetic were beginning to wear off. Aseptic technique was not required, and postoperative mortality was low.

A total of 160 cockroaches were inoculated with methylcholanthrene and 108 with talc. In half, the incision through the body wall was made on the ventral rather than the dorsal surface to avoid injuring the recurrent or other large nerves that supply the intestinal tract. An additional 24 insects were subjected to the complete operation except that neither methylcholanthrene nor talc was introduced when the probe entered the abdominal cavity. Another 24 roaches were decapitated before the other experimental procedures were carried out.

Of the 316 animals used, 170 were examined histologically. These had died or were killed at the following intervals after operation: less than 1 day, 8; 1-10 days, 54; 11-20 days, 20; 21-40 days, 21; 41-80 days, 10; 81-160 days, 26; 160-261 days, 11; 261-365 days, 20. A 1-cm.-long segment of the abdomen containing the injured region was excised, and the intact dorsal or ventral chitinous plate was carefully dissected free of the underlying soft tissues. The latter, still attached to the plate bearing the initial site of incision, were then placed in 10% formalin. Subsequently 15 to 20 serial cross sections were made at four levels and stained with hematoxylin and eosin. Additional sections were stained with Mallory's trichrome, phosphotungstic acid-hematoxylin, and silver reticulin stains.

RESULTS

At the time of operation a drop or two of clear, pale yellow hemolymph escaped from the wound. Smears of the hemolymph prepared with Wright's stain reveal a moderate number of cells. These are round, oval, or fusiform, with large spherical nuclei and variable amounts of basophilic cytoplasm (Fig. 1*A*). The chromatin of the nuclei is finely granular and in staining reaction ranges from deep basophilia to pronounced acidophilia. None of the cells has an oxygen-carrying pigment, nor

7. Raymond, P. E.: Prehistoric Life, Harvard University Press, Cambridge, 1947.

8. This chow is produced by the Ralston Purina Company, St. Louis.

can the cells be classified into groups as are the white blood corpuscles of most vertebrates. Although several distinct cell types are recognized among insectan hemocytes,[9] those found in the circulating hemolymph of the roach probably represent different stages of maturation of a single stem cell.[10] When hemocytes are seen in tissue spaces, e. g., between muscle bundles (Fig. 1*B*), they do not show the fusiform shape nor the variability of staining qualities noted in the smear preparation.

Coagulation of the shed hemolymph occurs within three to five minutes and is characterized by early agglutination of the hemocytes. The latter phenomenon is readily seen in the clumping of the cells during preparation of the conventional smear. Although earlier studies [11] suggest that the plasma exhibits little change during clotting, Ermin [10] describes the appearance of a plasma coagulum in addition to a clumping of the hemocytes. Recently Grégoire [12] has observed that among the hemocytes of many insects are some that are distinguished by the hyaline appearance of the cytoplasm under phase contrast illumination. Alterations of these hemocytes are followed by various degrees of plasma coagulation.

Despite the fact that hemolymph was lost during the operation and that large numbers of hemocytes accumulated about the site of injury, mitosis was not observed in subsequent smears or tissue sections. Changes of the cell complex similar to those described by Taylor [13] as following starvation or dehydration were not seen. Yeager and Tauber [14] have estimated the total number of hemocytes in a roach at about one million cells. This suggests that a large number of hemocytes can be immobilized at the site of injury without notably affecting the number of cells distributed elsewhere in the body. No special hematopoietic organs are present in the roach; hemocytes attach themselves to the surface of the viscera, tracheae, and the walls of vascular channels (Fig. 1*B*), where they undergo mitosis.[15] This was demonstrated by Ermin, who found many of the small immature hemocytes in process of division after injecting colchicine into roaches. During ecdysis (molting) marked proliferation of the hemocytes is a normal occurrence.[16] Multinucleate hemocytes are occasionally seen in the roach, but their significance is uncertain.[17]

9. Wigglesworth, V. B.: Principles of Insect Physiology, London, Methuen & Co., Ltd., 1939.

10. Ermin, R.: Über Bau und Funktion der Lymphocyten bei Insekten (Periplaneta Americana), Ztschr. Zellforsch. u. mikr. Anat. **29**:613-669, 1939.

11. Yeager, J. F.; Shull, W. E., and Farrar, M. D.: On the Coagulation of Blood from the Cockroach, Periplaneta Orientalis, with Special Reference to Blood Smears, Iowa State Coll. J. Sc. **6**:325-345, 1932.

12. Grégoire, C.: Blood Coagulation in Arthropods: II. Phase Contrast Microscopic Observations on Hemolymph Coagulation in 61 Species of Insect, Blood **6**:1173-1198, 1951.

13. Taylor, A.: Experimentally Induced Changes in the Cell Complex of the Blood of Periplaneta Americana, Ann. Entomol. Soc. America **28**:135-145, 1935.

14. Yeager, J. F., and Tauber, O. E.: Determination of Total Blood Volume in the Cockroach Periplaneta Fuliginosa, with Special Reference to Method, Ann. Entomol. Soc. America **25**:315-327, 1932.

15. Yeager, J. F., and Hendrickson, G. O.: Circulation of Blood in Wings and Wing Pads of the Cockroach, Periplaneta Americana, Ann. Entomol. Soc. America **27**:257-272, 1934.

16. Wigglesworth, V. B.: The Physiology of the Cuticle and of Ecdysis in Rhodnius Prolixus; with Special Reference to the Function of the Oenocytes and the Dermal Glands, Quart. J. Micr. Sc. **76**:269-318, 1933.

17. Tauber, O. E., and Griffiths, J. T., Jr.: Multinucleate Hemocytes in the Roach, Blatta Orientalis, Tr. Am. Micr. Soc. **42**:91-93, 1943.

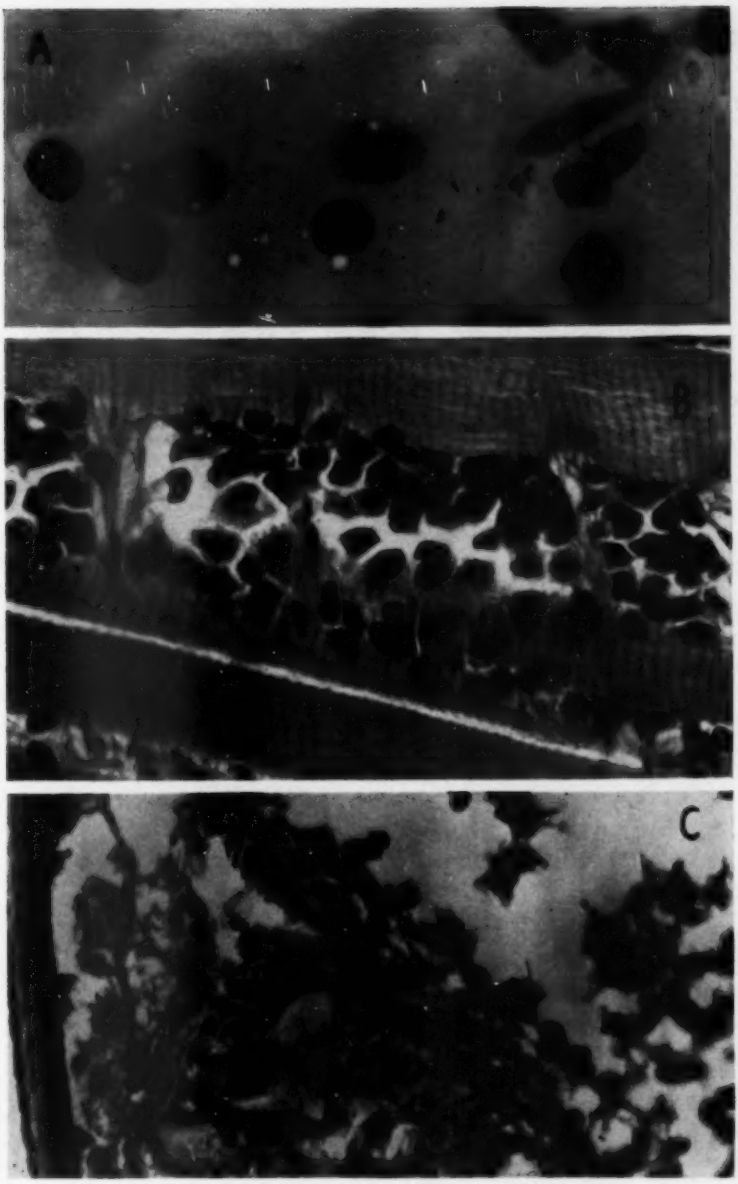

Fig. 1.—*A*, hemocytes in a smear preparation of the hemolymph. Note the diffuse finely granular chromatin in the nuclei and the variable amount of basophilic cytoplasm. Wright's stain; × 810.

B, hemocytes in a "vascular" channel between thoracic muscle bundles. Hematoxylin-eosin stain; × 450.

C, stellate hemocytes accumulating about a mass of talc seven hours after it was introduced into the body cavity. On the extreme left is the cuticle; the adjacent hypodermis shows early degenerative changes. Hematoxylin-eosin stain; × 450.

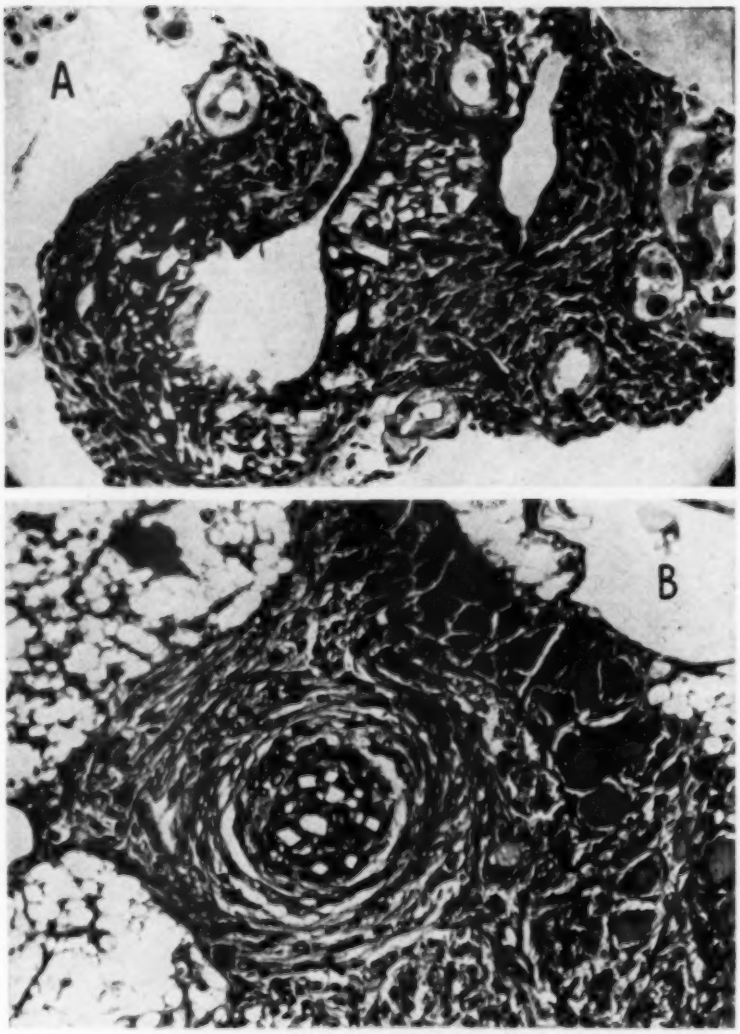

Fig. 2.—*A*, hemocytes surrounding rectangular crystals of methylcholanthrene 70 hours after operation. The two large spaces are the lumina of tracheae. Embedded in the mass of fusiform hemocytes are several Malpighian tubules. Hematoxylin-eosin stain; × 200.

B, encapsulation of a mass of methylcholanthrene crystals after nine days. Note the increased flattening and beginning degeneration of the innermost layer of hemocytes. Hematoxylin-eosin stain; × 230.

Seven hours after the operation hemocytes are seen accumulating about the methylcholanthrene or the talc (Fig. 1*C*). Most of the cells retain the appearance of the circulating hemocytes, although pseudopodia have now become prominent. After 45 hours, many of the hemocytes have fused to form a syncytial network; others, especially when they lie in the interstices of tissues, appear distinctly elongated. Three days after injury this elongation is notable even in accumulations of hemocytes lying free in the body cavity (Fig. 2*A*), where they begin to resemble connective tissue, although fibrils and tracheoles are absent. Thereafter the innermost layers of hemocytes are surrounded by additional hemocytes that arrange themselves in concentric layers forming a veritable capsule about the talc or the methylcholanthrene (Fig. 2*B*). Cameron [18] and Iwasaki [19] have observed similar changes in caterpillars.

Tracheoles appear after two to three weeks, lending the lesion an appearance similar to that of a granuloma in man (Fig. 3). This resemblance is given greater meaning by the fact that the tracheoles are analogous to mammalian arterioles and capillaries, for they carry oxygen, albeit in the form of air, directly to the tissues. A similar relationship of collections of hemocytes and tracheae was observed by Thorpe [20] in his study of the reaction of an insect host to its insect parasite. Careful inspection of many "granulomata" failed to provide convincing evidence that the tracheal cells form any part of the pseudotissue other than the tracheal walls.

Within 8 to 14 days the cells at the center of the nodule degenerate; the nuclei are flattened and pyknotic, the cytoplasm dense and hyalinized. Occasionally these cells become orange brown owing to the oxidation of liberated melanogens by the trypsinase present in the hemolymph.[10]

The relation of the pseudotissue to true connective tissue is subject to the same uncertainty that has plagued students of these cells in vertebrate animals. Lazarenko,[21] using larvae of the rhinocerous beetle, was particularly concerned with this problem. He concluded that when hemocytes have united to form a syncytium the component spindle-shaped cells may be identified as desmoblasts. In the present experiments fibrils occasionally appeared within the cytoplasm of the elongated hemocytes (Fig. 4*A*). Nevertheless, connective tissue as we are accustomed to find it in vertebrates is rarely seen among insects.[10] In the cockroach a tissue that may be so classified is found between the epithelial folds of the intestinal tract; Riedel [22] has recently identified delicate strands of connective tissue in the basement membrane of the midgut of the grasshopper. Lartschenko,[23] studying the cellular response of caterpillars to eggs of parasitic wasps, stated that the cells

18. Cameron, G. R.: Inflammation in the Caterpillars of Lepidoptera, J. Path. & Bact. **38**:441-466, 1934.

19. Iwasaki, Y.: Sur quelques phénomènes provoqués chez les chenilles de papillons par l'introduction de corps étrangers, Arch. anat. micr. **23**:319-346, 1927.

20. Thorpe, W. H.: On a New Type of Respiratory Interrelation Between an Insect (Chalcid) Parasite and Its Host (Coccidae), Parasitology **28**:517-540, 1936.

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22. Riedel, F. A.: Connective Tissue Pattern in the Ventriculus of Certain Lubber Grasshoppers, Ann. Entomol. Soc. America **39**:298-303, 1946.

23. Lartschenko, K.: Die Unempfänglichkeit der Raupen von Loxostege sticticalis und Pieris brassicae gegen Parasiten, Ztschr. Parasitenkunde **5**:679-707, 1933.

which surround and ultimately encapsulate the eggs are derivatives of the primitive mesenchyme. She expressed the belief that these usually become fat cells but in the presence of a foreign body take on the function of connective tissue. No such transformation was observed in the cockroach. Because of the pronounced phagocytic powers of the hemocytes [24] and their ability to form at least a pseudotissue, Stern [25] suggested that in insects they represent a reticuloendothelial system in its lowest stage of development.

While this reaction to methylcholanthrene or talc is occurring within the body cavity of the roach, the hemocytes plugging the initial wound in the body wall have

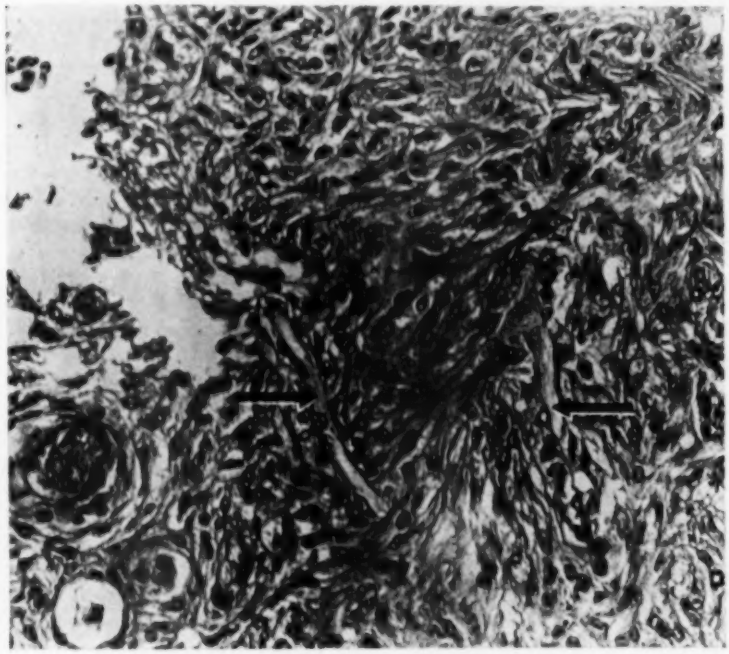

Fig. 3.—An interlacing mass of hemocytes and tracheoles (arrows) 80 days after injury. The hemocytes have a moderately abundant pale-staining granular cytoplasm. Hematoxylin-eosin stain; × 310.

been undergoing similar changes. After 8 to 10 days the cells comprising the single-layered cuboidal epithelium that lies beneath the cuticle enlarge and begin to migrate from the margin of the wound into or beneath the mass of hemocytes (Fig. 4*B*). Occasionally these cells are seen to divide, although usually mitosis occurs in cells

24. Bettini, S.; Sarkaria, D. S., and Patton, R. L.: Observations on the Fate of Vertebrate Erythrocytes and Hemoglobin Injected into the Blood of the American Cockroach, Science **113**:9-10, 1951.

25. Stern, N. A.: Formation of a Peculiar Parenchymatous Tissue in Musca Domestica upon Feeding with Ammonia Carmin, Bull. Acad. Sc. USSR 1347-1370, 1931 (Russian); Biol. Abstr. **8**:2035, 1934.

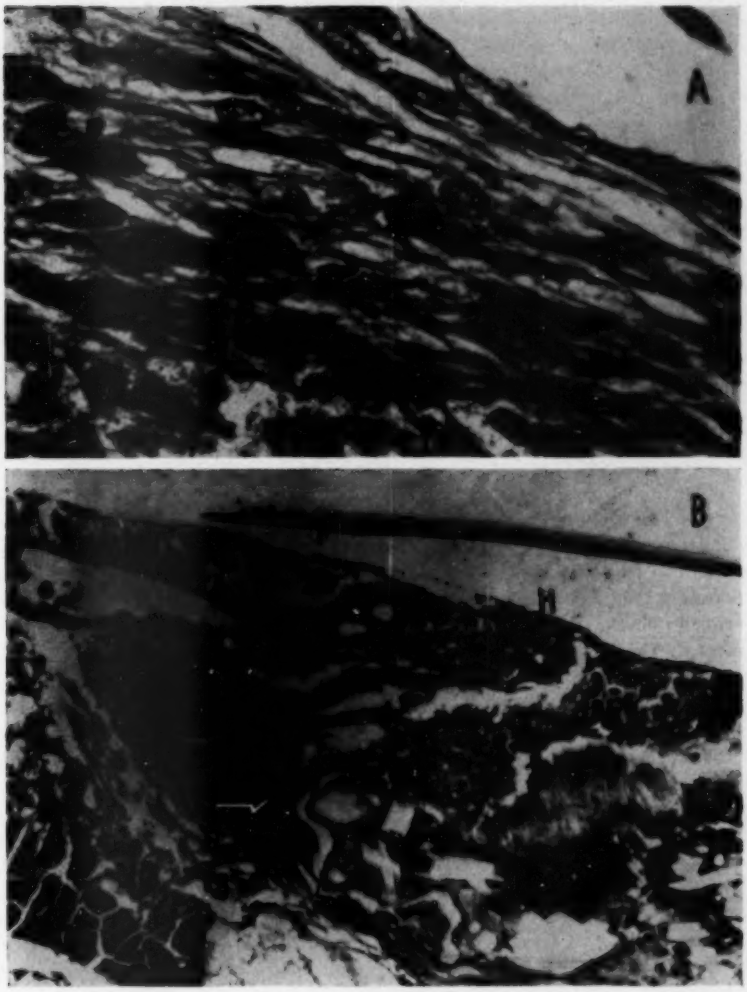

Fig. 4.—*A*, fibrils within the cytoplasm of hemocytes 105 days after the introduction of talc. Mallory's trichrome stain; × 810.

B, single layer of epithelium (arrow) growing downward and around a mass of hemocytes and methylcholanthrene 15 days after injury. The encircling cells arise from the forward margin (*H*) of the hypodermis. Hematoxylin-eosin stain; × 235.

that are some distance from the advancing edge of the epithelium. Wigglesworth,[25b] studying wound healing in the insect Rhodnius prolixus, pointed out that there is an intermediate zone depleted of cells by the migration of the epithelium, where mitotic figures are subsequently most numerous.

In a recent report on wound healing following focal burns in the epidermis of caterpillars, Kühn [26] noted the formation of polypoid giant cells similar to those previously observed by Wigglesworth in the wounds of starved or burned insects. In the present experiments, although methylcholanthrene was in direct contact with the regenerating epithelium in several instances, giant cells with large or abnormal nuclei were not seen. On occasion a small multilayered nodule of epithelial cells was present at the base of the wound, but these foci of hyperplasia were found only in the early stages of wound healing; there was no evidence of subsequent autonomous growth.

When talc or methylcholanthrene was introduced into the body cavity, various organs occasionally suffered injury. In the case of female roaches the eggs were occasionally injured, the yolk escaping into the abdominal cavity. This led to a very intense response, with hemocytes accumulating in large masses about the extravasated yolk (Fig. 5*A*). Rarely the follicle cells of such ova enlarged to form giant cells with bizarre, irregular, pyknotic nuclei (Fig. 5*B*), but nothing resembling a neoplasm was seen.

Some of the cells of the fat body, which occupies the interstices between the other viscera, were invariably injured during operation. The cytoplasm became vacuolated and the nuclei pyknotic. Often the cells were reduced to globules of melanin-like pigment surrounded by encapsulating hemocytes. No proliferation or abnormal mitotic figures like those observed by Mercier [27] in the fat cells of microsporidian-infested roaches were seen, nor did talc or methylcholanthrene, when in contact with these cells, elicit such a response.

The tracheae are often injured during operation. Dissection of the abdominal viscera under moderate magnification reveals that even the larger tracheae are easily torn. After injury, inspissated fluid (probably hemolymph) and hemocytes appear in the lumen (Fig. 6); large numbers of hemocytes often surround the tracheae. Similar changes were occasionally found in normal roaches by Sanford,[28] who suggested that during molting slight breaks might occur in the tracheae, allowing an influx of hemolymph. In the present experiments the lumina of the tracheae were sometimes completely occluded by hemocytes, hemolymph, proliferating tracheal cells, and irregular masses of structureless material that had the staining properties of chitin. That injury of the tracheae may be followed by multiplication of tracheal cells, and increased secretion of chitin was demonstrated by Beard,[29] who showed that when cactus spines punctured tracheae of the squash bug they were surrounded by a sheath of proliferating, chitin-secreting tracheal cells.

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26. Kühn, A.: Wundheilung und Riesenzellenbildung bei Ptychopoda seriata, Ztschr. Naturforsch. **4b**:104-108, 1949.

27. Mercier, L.: Néoplasie du tissu adipeux chez des Blattes (Periplaneta orientalis) parasitées par une Microsporidie, Arch. Protistenkunde **11**:372-381, 1908.

28. Sanford, E. W.: Experiments on the Physiology of Digestion in the Blattidae, J. Exper. Zool. **25**:355-411, 1918.

29. Beard, R. L.: On the Formation of the Tracheal Funnel in Anasa Tristis Induced by the Parasite Trichopoda Pennipes, Ann. Entomol. Soc. America **35**:68-74, 1942.

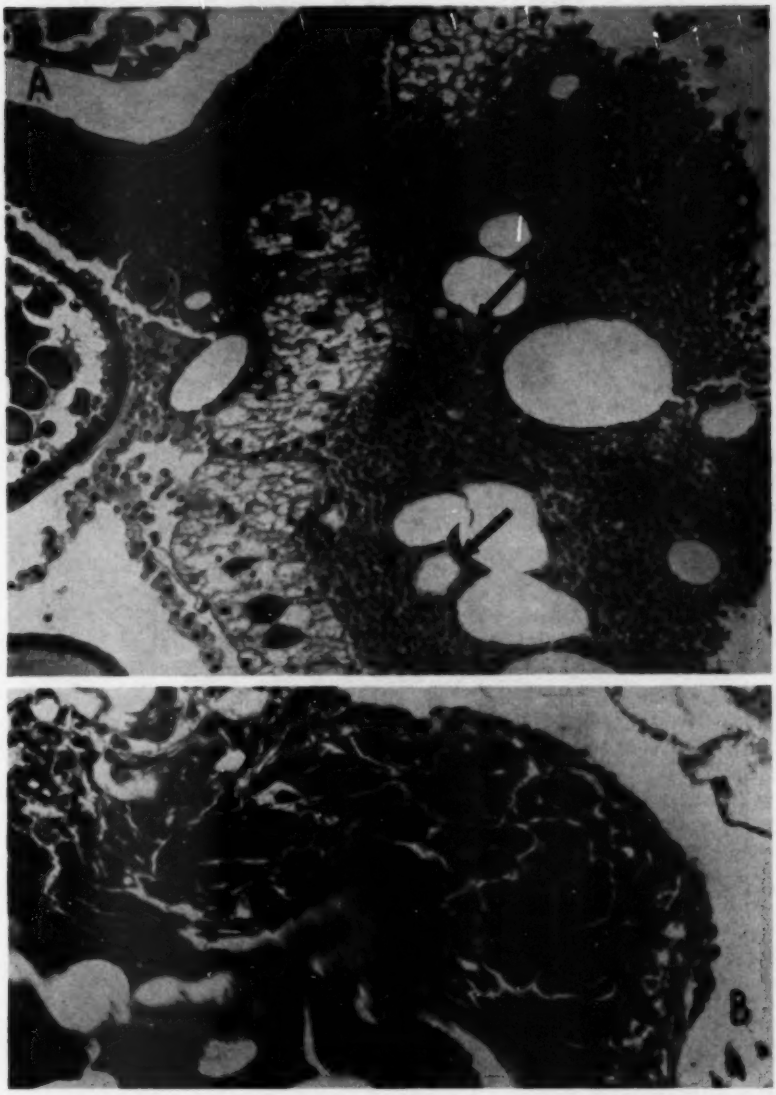

Fig. 5.—In *A*, at the left, are portions of three oöcytes, each surrounded by a layer of cuboidal follicle cells. Between the oocytes and the fat cells is a dense mass of hemocytes engulfing extravasated yolk (arrows) 39 days after injury of the oöcytes. Hematoxylin-eosin stain; × 125.

B, collection of giant follicle cells at the periphery of an injured oöcyte. Hematoxylin-eosin stain; × 250.

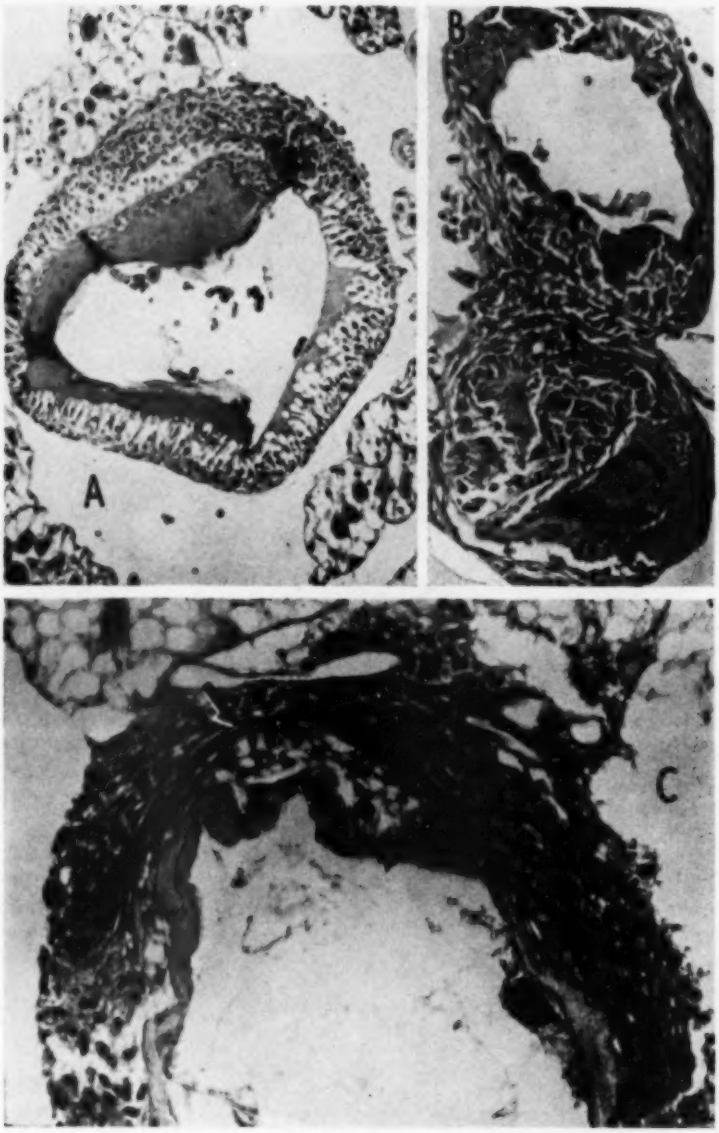

Fig. 6.—*A*, hemocytes and hemolymph within a large trachea which has been injured; hemocytes also surround the damaged wall; × 95.

B, two damaged tracheae; × 190. The lumen of the lower one is occluded by proliferating tracheal cells, chitin, and a few hemocytes. In the upper one, a bit of chitinous "intima" is seen free in the lumen. *C*, portion of injured trachea showing the arciform thickening or "spiral thread" (arrow) of the chitinous intima. Outside the latter is an accumulation of hemocytes; lining the lumen are additional hemocytes and chitin. Hematoxylin-eosin stain; × 300.

The importance of the tracheae for transporting oxygen to the tissues is well recognized, and details of the mechanism have been reviewed.[30] It is notable that in the present series of experiments the most extensive collections of hemocytes were found about the midgut or ceca (Fig. 7). Often there was an associated plugging of the tracheae and localized necrosis of the epithelium that resembled an infarct in mammals (Fig. 8*A*). Similar results were obtained in a group of roaches in which the same operative procedures had been carried out except that neither methylcholanthrene nor talc was introduced when the probe was inserted into the body cavity. Care was taken to avoid injuring the recurrent or other major nerves.

When a cockroach is drowned in India ink, the larger tracheae and some of their small branches become filled with ink. On dissection the tracheae are clearly visible as black threads against the white background of the fat body and other viscera. The very rich tracheal supply of the midgut is at once apparent, indicating that these tissues probably have a high oxygen demand and are readily damaged by any diminution of the supply. When studying digestion in the tsetse fly, Wigglesworth [31] observed that ingested blood from the lumen of the midgut entered the tracheoles. Commenting on the abundant tracheal network that supplies the intestinal epithelium he wrote: "In the feeding of *Glossina* nature has performed the experiment of injecting the tracheal system from its periphery and in the process has revealed an amazing skein of tracheoles not only in the subepithelial layer, but within the cytoplasm of the cells." This plugging of the tracheoles with ingested blood may become extreme and lead to necrosis and dissolution of the epithelium; here again the lesion bears some resemblance to the mammalian infarct.

The correlation of tracheal obstruction and necrosis of the midgut epithelium may be valid, but it must be pointed out that normally these cells degenerate and are replaced by others derived from groups of less-differentiated epithelial cells located near the basement membrane. Gresson [32] observed that after long periods of secretion the greater part of the epithelial lining of the ceca and of the anterior portion of the midgut degenerates more or less simultaneously. Nevertheless, the fact remains that in the present experiments the greatest accumulations of hemocytes are found about areas of necrosis in the midgut associated with damaged tracheae. Talc or methylcholanthrene could seldom be demonstrated in these regions.

Occasionally the epithelium at the periphery of the necrotic areas in the midgut was distorted. This was particularly true of the nests of "regenerative" cells, where mitosis and migration occurred simultaneously (Fig. 8*B*). A similar change was described by Ries [33] for the midgut of the mealworm when bits of transplanted tissue lay near the epithelium. After section of the recurrent nerve of a tropical roach Scharrer [34] noted ". . . the transition of the normal columnar epithelium

30. Wigglesworth, V. B.: The Respiration of Insects, Biol. Rev. **6**:181-220, 1931. Krogh, A.: The Comparative Physiology of Respiratory Mechanisms, Philadelphia, University of Pennsylvania Press, 1941.

31. Wigglesworth, V. B.: Digestion in the Tsetse Fly: A Study of Structure and Function, Parasitology **21**:288-321, 1929.

32. Gresson, R. A. R.: The Cytology of the Midgut and Hepatic Ceca of Periplaneta Orientalis, Quart. J. Micr. Sc. **77** N.S.:317-334, 1935.

33. Ries, E.: Experimentelle Symbiosestudien: I. Mycetomtransplantionen, Ztschr. Morph. u. Oekol. d. Tiere **25**:184-234, 1932.

34. Scharrer, B.: Experimental Tumors After Nerve Section in an Insect, Proc. Soc. Exper. Biol. & Med. **60**:184-189, 1945.

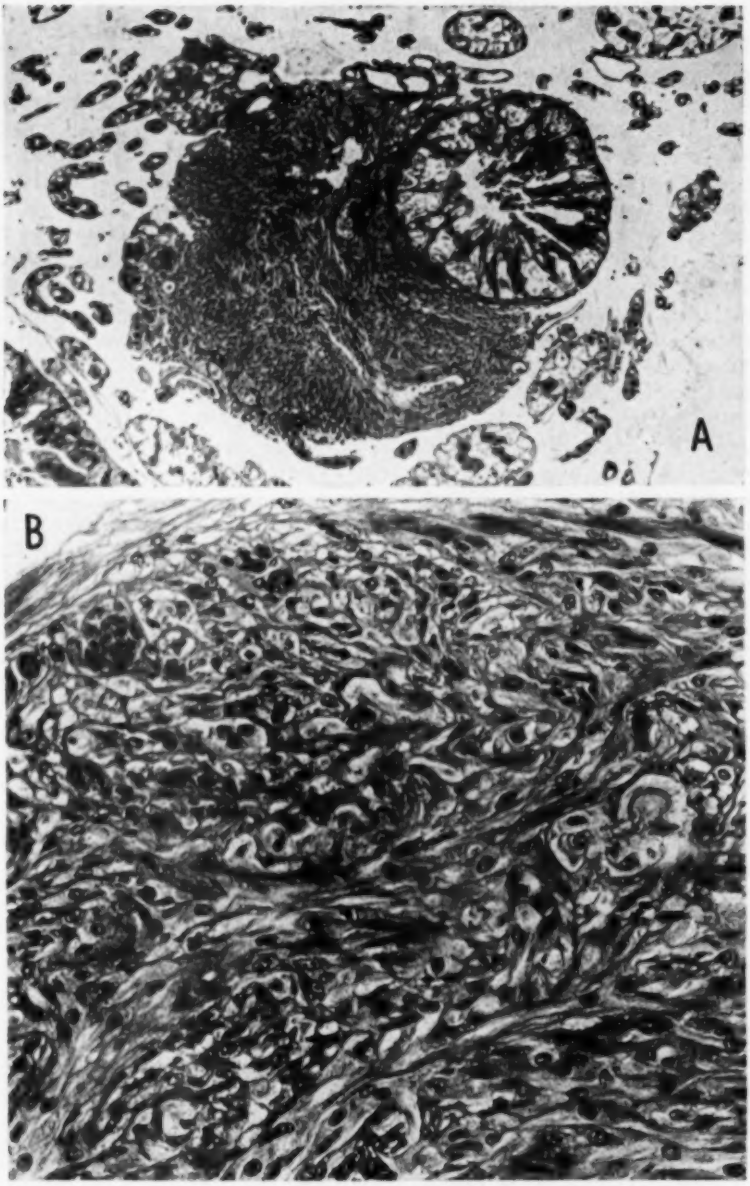

Fig. 7.—*A*, massive collection of hemocytes partly surrounding one of the ceca 85 days after methylcholanthrene was introduced into the body cavity. Hematoxylin-eosin stain; × 30.

B, detail of *A*. Note the interlacing bundles of fibrils and the epithelioid character of the hemocytes. Hematoxylin-eosin stain; × 30.

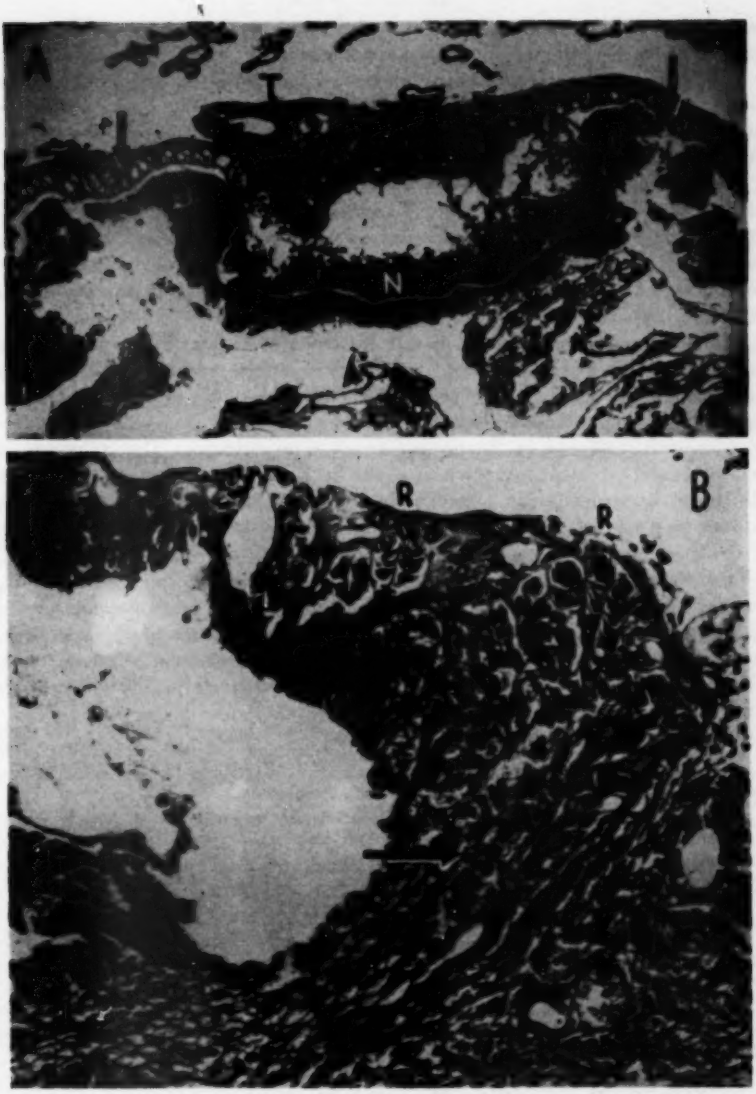

Fig. 8.—*A*, necrosis of the epithelium of the midgut associated with partial occlusion of tracheae and accumulation of hemocytes 79 days after operation. Arrows indicate normal epithelium; *N*, necrotic epithelium; *T*, damaged tracheae. Hematoxylin-eosin stain; × 120.

In *B*, elements from the islets of reserve or regenerating cells (*R*) have replaced (arrow) the normal columnar epithelium of a cecum 40 days after injury; in the lower right, hemocytes have surrounded two Malpighian tubules. Hematoxylin-eosin stain: × 175.

of the midgut . . . into [an] irregular, multilayered tumor mass . . ." This would imply that the epithelium itself had become neoplastic.

The interesting work of Scharrer on tumors induced in the salivary gland and foregut of an insect by nerve severance,[35] as well as her review of tumors found in the invertebrates,[36] reflects the growing interest of biologists in these problems.[37] Whether the lesions reported by Scharrer are true neoplasms in the sense in which the term is used to denote tumors of vertebrates is uncertain. In several of the tumors hemocytes appear to figure prominently,[38] suggesting that the epithelial proliferation is hyperplasia accompanying a reaction to injury. The injury may be sought in stasis following nerve section and the resultant accumulation of food and secretions.[39] A definitive histopathologic study of these lesions will be awaited with interest.

Methylcholanthrene has been used in the study of insects (Drosophila) chiefly to determine its mutagenic activity.[40] The systemic effect of other chemical carcinogens, namely, the azo dyes, has been studied on roaches.[41] No tumors following the use of these agents have been reported, nor were any lesions that could be identified as neoplasms produced in the present series of experiments. The collections of hemocytes and accompanying tracheae somewhat resemble the granulomas occasionally found in man after an operation in which talc has been used as a dusting powder on the surgeon's gloves.[42] Typical foreign-body giant cells, so characteristic of the lesion in man, were not seen in the roach.

Recent work on insect hormones indicates that the brain [43] and the neighboring corpora allata [9] are intimately concerned with molting and postembryonic growth of insects. In lepidoptera Williams [44] has demonstrated that the prothoracic glands secrete the growth-and-differentiation hormone when stimulated to do so by the brain. However, there is yet no evidence that prothoracic glands occur in the

35. Scharrer, B.: Gastric Cancer Experimentally Induced in Insects by Nerve Severance, J. Nat. Cancer Inst. **10**:375-376, 1949; Tumor Mortality and Sex in Leucophaea Maderae. Anat. Rec. **105**:624, 1949. Wilson, V. T., and Scharrer, B.: Fat Metabolism in Tumor Bearing Insects (Leucophaea Maderae), ibid. **105**:625, 1949. Scharrer.[34]

36. Scharrer, B., and Lochhead, M. S.: Tumors in the Invertebrates: A Review, Cancer Res. **10**:403-419, 1950.

37. Gersch, M.: Zellentartung und Zellwucherung bei wirbellosen Tieren, Arch. Geschwülstforsch. **3**:1-18, 1951.

38. Scharrer,[34] Figs. 6 and 7.

39. Snipes, B. T., and Tauber, D. E.: Time Required for Food Passage Through the Alimentary Tract of the Cockroach, Periplaneta Americana, Linn, Ann. Entomol. Soc. America **30**:277-284, 1937.

40. Demerec, M.: Production of Mutations in Drosophila by Treatment with Some Carcinogens, Science **105**:634, 1947. Burdette, W. J.: Lethal Mutation Rate in Drosophila Treated with 20-Methylcholanthrene, ibid. **112**:303-305, 1950.

41. Noland, J. L., and Baumann, C. A.: Effects of Certain Azo Dyes upon the Cockroach, Blatella Germanica, Proc. Soc. Exper. Biol. & Med. **71**:365-368, 1949.

42. Seelig, M. G.; Verda, D. J., and Kidd, F. H.: The Talcum Powder Problem in Surgery and Its Solution, J. A. M. A. **123**:950-954, 1943.

43. Williams, C. M.: Physiology of Insect Diapause: The Role of the Brain in the Production and Termination of Pupal Dormancy in the Giant Silkworm Platysamia Cecropia, Biol. Bull. **90**:239-243, 1946

44. Williams, C. M.: Biochemical Mechanisms in Insect Growth and Metamorphosis, Federation Proc. **10**:546-552, 1951.

cockroach.[45] Pflugfelder [46] has shown that excision of the corpora allata of the walking-stick insect (Dixippus) produces degeneration of the fat cells, cyst formation, and the development of multinucleate giant cells.

On the basis of these findings and the new discovery that the anterior pituitary-adrenal complex is related to inflammation in mammals, 24 roaches were decapitated, depriving them of brain and corpora allata. While they were still anesthetized, talc or methylcholanthrene was introduced into the abdominal cavity and the insects were returned to a moist chamber. The entire procedure was accompanied by little loss of hemolymph. The animals were killed at intervals of 24 hours, the maximal survival period was seven days. In three instances death was due to bacteremia. Neither a qualitative nor a quantitative difference could be demonstrated between the cellular response observed in the decapitated roaches and that of the normal roach. The accumulation of hemocytes and the formation of a pseudotissue were unaltered.

SUMMARY

Methylcholanthrene and talc elicit similar responses in the body cavity of the cockroach; both are quickly surrounded by large numbers of hemocytes.

After two to three days the hemocytes become elongated and form a syncytium that somewhat resembles mammalian connective tissue. This appearance is enhanced by the fibrils that subsequently develop, probably within the cell cytoplasm. Numerous tracheae penetrate the larger masses of hemocytes.

Occasionally large tracheae are damaged during operation, and their lumina become filled with hemolymph, hemocytes, and chitin. This occlusion of the tracheae may interfere with oxygen exchange in the tissues and lead to "infarction." The intestinal epithelium, which is normally richly supplied with tracheae, is particularly susceptible to this form of injury.

The accumulation of hemocytes about the damaged intestine may be so great that it resembles a neoplasm. However, there is no evidence of autonomous growth; at best the lesion may be compared with the mammalian granuloma.

Removal of the brain and corpora allata by decapitation failed to alter the extent or the character of the response to methylcholanthrene or talc.

45. Williams, C. M.: Physiology of Insect Diapause: III. The Prothoracic Glands in the Cecropia Silkworm, with Special Reference to Their Significance in Embryonic and Post-embryonic Development, Biol. Bull. **94**:60-65, 1948.

46. Pflugfelder, O.: Atypische Gewebsdifferenzierungen bei Stabheuschrecken nach experimentaller Störung der inneren Sekretion, Ztschr. Krebsforsch. **56**:107-120, 1948.

News and Comment

New Officers.—The annual meeting of the American Association of Pathologists and Bacteriologists was held in New York, April 10 to 12, 1952. The following officers were elected: Dr. William H. Feldman, president; Dr. James B. McNaught, vice-president; Dr. Alan R. Moritz, secretary; Dr. Sidney Farber, treasurer; Dr. Granville A. Bennett, incoming member of Council; Dr. Paul Klemperer, member of council to fill unexpired term of Dr. Tracy B. Mallory. Dr. J. Lowell Orbison, assistant secretary; Dr. William A. Meissner, assistant treasurer. The next meeting of the Association will be held in St. Louis, on April 2, 3, and 4, 1953. The topic for the symposium is "The Modification of Structural Changes in Infectious, Neoplastic, and Other Diseases Following Use of Modern Chemotherapeutic Agents," with Dr. Sidney Farber and Dr. William H. Feldman to act as referees.

Cancer Research.—The Sixth Annual Symposium on Fundamental Cancer Research sponsored by the University of Texas, M.D. Anderson Hospital for Cancer Research, was held in Houston, Texas, April 25 and 26, 1952. Meeting jointly with the South Central Region College of American Pathologists, the Texas Society of Pathologists, and the Houston Society of Pathologists, the Symposium drew an attendance of about 300 physicians from 22 states and Mexico. The meeting was divided into three major sections: a panel on nutritional factors in cancer research, a cancer pathology and radiology conference, and a session devoted to fundamental cancer research.

A highlight of the Symposium was the presentation of the annual Bertner Foundation Award posthumously to Dr. George Milton Smith in recognition of his lifetime achievements as an organizer and administrator of cancer research. The annual Bertner Lecture was given by Dr. Stanhope Bayne-Jones, of New York Hospital-Cornell Medical Center; his subject was "Aspects of Cancer Research in the United States."

Death Notice.—Dr. Douglas Symmers, director of the Pathology Service, Department of Hospitals, New York City, died on April 19, 1952.

Dr. Symmers graduated from Jefferson Medical College in 1901. In 1916 he was appointed director of laboratories of Bellevue and Allied Hospitals and professor of pathological anatomy at Bellevue Medical College. The latter position was held continuously until his retirement in 1941. When the municipal hospitals of New York City were consolidated under a Department of Hospitals in 1929, Dr. Symmers was appointed general director of the Laboratories of Pathology. He remained active in this position until his death.

Dr. Symmers belonged to the New York Academy of Medicine, the New York Pathological Society, the American Association of Pathologists and Bacteriologists, and the Hodgkin's Disease Research Foundation. His interests were concentrated in fields of pathologic anatomy and, in particular, in disorders of lymphoid tissue. One of these disorders, giant follicular lymphadenopathy, is frequently referred to by the eponyms Symmer's disease and Brill-Symmers disease.

Books

TUMORS OF THE EYE. By Algernon B. Reese, M.D., D.Sc. (Hon.), attending ophthalmologist and pathologist, Institute of Ophthalmology, Presbyterian Hospital, New York; ophthalmologist to Memorial Center for Cancer and Allied Diseases, New York; clinical professor of ophthalmology, College of Physicians and Surgeons, Columbia University. Pp. 574, with 511 illustrations, 122 in full color. Price $20. Paul B. Hoeber, Inc. (Medical Book Department of Harper & Brothers), 49 E. 33d St., New York 16, 1951.

This new monograph presents a comprehensive and well-integrated discussion of the pathologic, diagnostic, and therapeutic aspects of tumors of the eye and adnexa. The author has drawn heavily upon his own experience for text and illustrative material but this has been favorably balanced by an adequate discussion of the pertinent literature. A valuable bibliography accompanies each chapter. The personal quality imparted to many of the pathologic and clinical interpretations and conclusions reveals the author's mature and critical judgment and imparts to the reader a feeling of contact with a dynamic and stimulating teacher.

The reviewer would question the author's positive acceptance of the Schwann cell as the histogenetic unit of tumors in neurofibromatosis. It is noted also that the author occasionally cites an obscure reference or preliminary report when more definitive publications of the same author are readily available (Chapter 17).

The chapters on retinoblastoma and pigmented tumors are particularly distinguished by scholarly discussion and a wealth of pathologic and practical clinical information.

There are 511 superb illustrations, including 122 photographs and drawings brilliantly reproduced in color. The numerous carefully selected photomicrographs are of excellent quality and adequately complement the text material. Pathologists will appreciate the low-power magnification of many of these photographic reproductions.

The publisher deserves commendation regarding the fine quality of craftsmanship expressed in a format which combines beauty and utility.

This monograph fills a real need in ophthalmologic literature and will be of great interest and value not only to the ophthalmologist but also to the pathologist in his study of neoplasms of the eye and its adjacent structures.

MUIR'S TEXT-BOOK OF PATHOLOGY. Sixth Edition. Revised by D. F. Cappell, M.D., F.R.F.P.S.G., professor of pathology, University of Glasgow; pathologist to the Western Hospitals Group, Glasgow consultant pathologist to the Western Regional Board. Pp. 1090, with 636 illustrations. Price $10. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1951.

"Muir's Pathology" for many years has been an old standby. In this, its sixth edition, Cappell has greatly enhanced its value by timely additions and enlargements of the original text. Diseases of the endocrine glands are brought up to date, and even the "adaptation syndrome" is discussed. In some instances, the author does not hesitate to give his personal view; thus, he ventures that so-called specific rheumatic pneumonia is probably mainly the result of fairly acutely developing left heart failure. Teratology, in general, is omitted. Yet a few congenital anomalies of the heart are discussed. However, today, when congenital cardiac anomalies have assumed great clinical importance, it would be desirable that more space be given to them. Continental nomenclature such as "histiomas," "cytomas" and terms like "cephaloid," though fully explained, make the reading at times somewhat difficult. There are 636 illustrations—all black and white—and most of them are of excellent quality. As a matter of fact, some of them are of such excellence that colored photographs are not missed.

This volume will be appreciated by the medical student who needs a clearly written, concise text. Also, practicing physicians who have occasion to look up the fundamentals of a new subject will find this book of use, since it is up to date. Young pathologists, however, will be disappointed. Though there is a short list referring to either old classic descriptions or more

recent fundamental work at the end of the book, there are no references appended to the chapters. The person who is an apprentice in a medical specialty should be trained not to get one expert's opinion, even if that one gives both sides of a questionable subject, but to obtain pertinent facts concerning disputable entities by reading reports of original research. The first step toward this education is to show the student where he can find some of the facts. In this, the volume is lacking.

ATLAS OF GYNECOLOGIC PATHOLOGY—COLOR FILM LIBRARY AND DESCRIPTIVE MANUAL. By Anthony V. Postoloff, M.D., pathologist and director of laboratories, Millard Fillmore Hospital, Buffalo; associate in pathology, University of Buffalo School of Medicine, and David H. Nichols, M.D., Captain, Medical Corps, United States Air Force; formerly chief resident obstetrician-gynecologist at Millard Fillmore Hospital, Buffalo. Pp. 90, with 100 color slides. Price $80. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1952.

The authors have prepared 100 Kodachrome® slides of gross and microscopic material representing lesions of the cervix, the endometrium, the myometrium, the fallopian tube, and the ovary. A text of 71 pages accompanies the slides. This contains a brief general discussion of the lesions of the female genital tract under each of the headings listed above. Each slide in turn is then briefly described and there is often a short history of the patient from which the specimen was obtained. There are two indices at the end of the text—one covering the subject matter, the other listing the Kodachrome® slides.

Two slides submitted to the reviewer were found to be of good quality. As an ancillary aid in teaching, such a set of Kodachromes® should be of distinct benefit. It cannot, however, take the place of fresh and fixed gross material nor of projected microscopic slides in which tissue relationships can be demonstrated.

THE INFLUENCE OF HORMONES ON ENZYMES. Edited by Roy Waldo Miner. Fifteen contributors. Annals of the New York Academy of Sciences, Volume 54, Article 4, Pages 531-728. Pp. 194, with numerous illustrations. The New York Academy of Sciences, 2 E. 63d St., New York 21, 1951.

Some fifteen papers presented at this symposium report effects of hormones on enzyme systems, either isolated to a greater or less extent or in tissues. The steroid group has been most studied of late: estrogens, androgens, and corticoids, together with the estrogen cognates, the stilbestrols. But it is recalled that 20 years ago Kendall suggested that thyroxin participated in oxidation-reduction systems, and that the problem of insulin action at the cell level is even older. Also considered here are actions of pituitary hormones and of epinephrine. Reading these papers one gets an impression, perhaps best summed up in a phrase of two of the present authors, relating to thyroxin: "as far as the mechanism of thyroxine's action on enzyme systems is concerned, everything remains to be discovered"—certainly an encouraging invitation to beginners. Stadie, evaluating the effects of hormones, particularly that of insulin, became convinced that "there was no unequivocal evidence that insulin had any effect upon any enzyme system when it existed in cell-free or homogeneous systems." Drs. E. D. Goldsmith and R. I. Dorfman, in their introduction to the symposium, postulate that hormones may exert their influence by a change in tissue enzyme levels, by functioning as a component of an enzyme system, or by effects on accelerators or inhibitors of enzyme systems; even these would seem not to exhaust the various possibilities. While generalizations have not yet been reached in this field, these papers represent valuable contributions, and emphasize the magnitude of this fundamental problem in cell physiology.

VERHANDLUNGEN DER DEUTSCHEN GESELLSCHAFT FÜR PATHOLOGIE. By E. Randerath. Pp. 387, with numerous illustrations. Price 50 German marks. Piscator-Verlag, Stuttgart 5, Eberhardstr. 10, 1951.

This volumes gives the papers and relevant discussions which were presented before the 34th meeting of the German Society of Pathology, held in Wiesbaden, Germany, April 20-23, 1950. The main theme was "Normal and Pathologic Morphology of the Peripheral Vegetative Nervous System." De Castro, E. Herzog, and F. Feyrter were the main discussants. The rest of the

program was devoted to a number of other pathologic subjects, principally to diseases of the meninges and the cardiovascular system. The only clinical pathologic discussion was an evaluation of blood sugar taken post mortem. In all, 48 papers were presented.

A Textbook of Orthopedics with a Section on Neurology in Orthopedics. By M. Beckett Howorth, M.D., clinical professor of orthopedic surgery, New York University Post-Graduate Medical School; formerly assistant clinical professor of orthopedic surgery, College of Physicians and Surgeons, Columbia University; associate attending surgeon, New York Orthopedic Hospital. In association with Fritz J. Cramer, M.D., Donovan J. McCune, M.D., A. Wilbur Duryee, M.D., J. William Littler, M.D., Walter A. Thompson, M.D. Pp. 1110. with 463 figures. Price $16. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1952.

This new textbook of orthopedics is well arranged by subject material and divisions and is easily read. In addition, it has a good index which should make it quite valuable as a reference book in orthopedics.

The discussion of each of the various orthopedic disorders contains a section of pathology. Frequently, the pathologic description is more involved with the gross than with the microscopic picture, but where necessary a description of the microscopic process is included. The specfiic sections of the book dealing with such pathologic entities as avascular necrosis, metabolic and endocrine disorders, and neurology in relation to orthopedic practice bring out the pathologic considerations very well. The section on tumors of the spine and extremities is particularly interesting. There probably will be some uncertainty in the minds of pathologists, first, as to the author's classification of the tumors, and, second, as to his method of categorizing. The author realizes this when he states, "No classification of tumors can be considered complete or perfect in the present state of our knowledge." The composition of tumors with respect to gross findings, roentgen interpretation, and actual histopathology is well correlated. The reproduction of both x-rays and photomicrographs has been accomplished satisfactorily.

In general, the clinical material is well correlated with the gross and microscopic pathologic changes, so that this book should be of interest to pathologists who are interested in orthopedic problems.

Lehrbuch der Röntgendiagnostik. By H. R. Schinz, W. E. Baensch, E. Friedl, and E. Uehlinger. Part 6: Innere Organe. Pp. 544, with 507 illustrations. Price 98 German marks. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart O; agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1952.

Die Krankhafte Blutdrucksteigerung. By Prof. Dr. L. Hantschmann, Leitender Chefarzt und Chefarzt der Inneren Abteilung der Städtischen Krankenanstalten Remscheid. Pp. 228, with 33 illustrations. Price 36 German marks. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart O; agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1952.

Recent Advances in Clinical Pathology. By S. C. Dyke, D.M. (Oxon.), F.R.C.P. (Lond.), pathologist, the Royal Hospital, Wolverhampton, and 32 contributing authors. Second edition. Pp. 575, with 37 illustrations, 26 figures, and 34 tables. Price $6. The Blakiston Company (Division of Doubleday & Company, Inc.), 1012 Walnut St., Philadelphia 5, 1952.

In the second edition "Recent Advances in Clinical Pathology" has been practically rewritten. About 25% of the subject matter which appeared in the first edition has been revised and enlarged in content in the present edition. This book contains four sections. Section I, on bacteriology, reviews the recent advances in regard to enteric infections and tuberculosis, the viruses, and the laboratory control of antibiotic therapy. Section II, on biochemistry, contains a chapter on liver function tests, which, although adequate, should have included the photometric method for the thymol turbidity test. The chapter on paper chromatography is an important contribution in the recent advances in clinical pathology. Perhaps the chapter on the electrolytes of body fluids is the most important for the clinical pathologist. Section III, on hematology,

stresses the Rh factor and the anemias. Section IV, on histology, edited by Robb-Smith, states in its introduction, "Diagnostic histology is the oldest branch of clinical pathology." In spite of the attempts that have been made to separate anatomic from clinical pathology, the present volume stresses the importance of histology as a clinical-pathological study. This is admirably illustrated by Gomori on histochemistry in diagnostic histology. The remainder of the section on histology reviews various recent aspects of pathological techniques and diagnosis in biopsies of endometrium, rectum and bladder, and skin, in studies of ovarian and testicular tumors, in neurosurgery, and in laboratory investigations of infertility. There is a special section on laboratory design and equipment. Although this topic is foremost in the minds of clinical pathologists, the reviewer wonders what particular importance this subject has in a volume on recent advances in clinical pathology.

Throughout this book one is constantly reminded of the sections that have been omitted in the present opus which appeared in the previous one, and the reader is told that it would be advisable to have both volumes at hand. Obviously, if newer editions appear in subsequent years, one would have to collect many volumes on recent advances in order to keep abreast of the progress made in clinical medicine and pathology. The second edition contains a great deal of information of value not only to the clinical pathologist but to the practicing clinician as well. In order that equilibrium may be maintained between the practicing physician and the practicing pathologist, it is suggested that both familiarize themselves with the recent advances as recorded in the present volume.

A Textbook of Pharmacology: Principles and Application of Pharmacology to the Practice of Medicine. By William T. Salter, M.D., professor of pharmacology, Yale University School of Medicine. Pp. 1240, with 284 figures. Price $15. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C.2, 1952.

Clinical Pathology of the Eye: A Practical Treatise of Histopathology. By Bernard Samuels, M.D., emeritus clinical professor of ophthalmology, Cornell University Medical College; consulting ophthalmologist, New York Hospital; acting advisory surgeon and consulting pathologist, New York Eye and Ear Infirmary, and Adalbert Fuchs, M.D., E. O. professor of ophthalmology, University of Vienna; consulting ophthalmologist and pathologist, New York Eye and Ear Infirmary. Pp. 420, with 418 illustrations, 191 in full color. Price $20. Paul B. Hoeber, Inc. (Medical Book Department of Harper & Brothers), 49 E. 33d St., New York 16, 1952.

The authors are accomplished clinical ophthalmologists, and in addition they are experienced in the pathology of their specialty. Their publication has the format of an atlas, with emphasis on pictures, but has a text that interprets the pathology for the clinician and correlates the clinical aspects with the pathology. The first 30 pages are devoted to a review of the general principles of pathology, including inflammation, degenerations, and changes due to age. The last are of especial importance in ophthalmology. From there, beginning with the cornea, the histologic elements of the eye are covered comprehensively, with a final chapter on tumors.

The illustrations are unique. They include not only black and white photomicrographs but a number of color plates. These are of some historic interest in addition to their illustrative value, being reproductions of drawings accumulated by Dr. Fuchs. There are 191 such colored drawings. As the authors state, it is at times easier to demonstrate a particular feature by a drawing than by a photomicrograph. This is particularly true where the illustrations are meant for the instruction of those who are not as accustomed to examining sections under the microscope as is the pathologist.

The section dealing with tumors might seem a bit limited in detail for the edification of the general pathologist, but the authors do not claim that their book is all-inclusive. It is really designed for the student of ophthalmology rather than the general pathologist or the highly specialized ophthalmic pathologist.

It has been the reviewer's experience that ophthalmologists as a class know more about the pathology of their specialty than do clinicians in other special fields. This volume will be an effective means for the trainee in ophthalmology who purposes to learn the pathology of the eye and a pleasant method of review for the practicing ophthalmologist.

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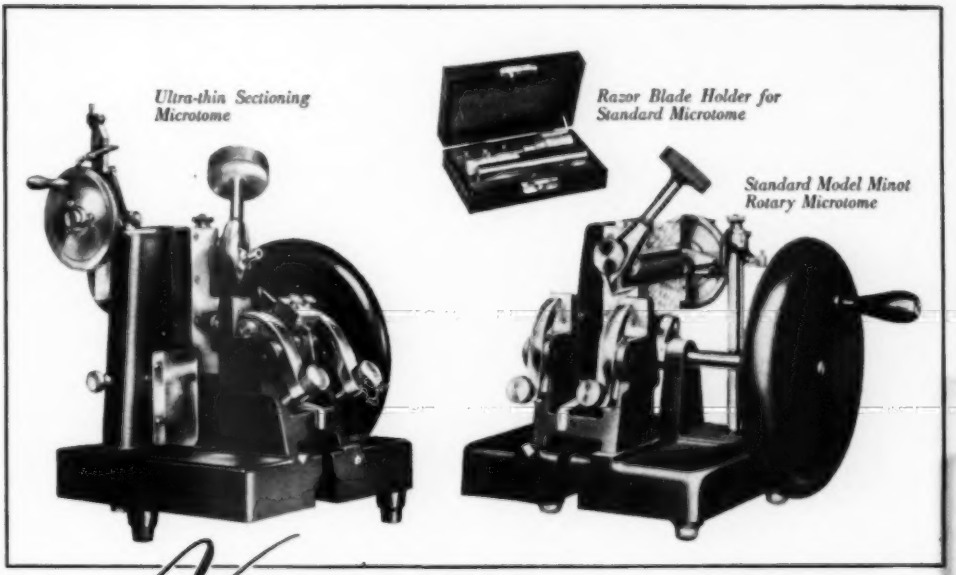

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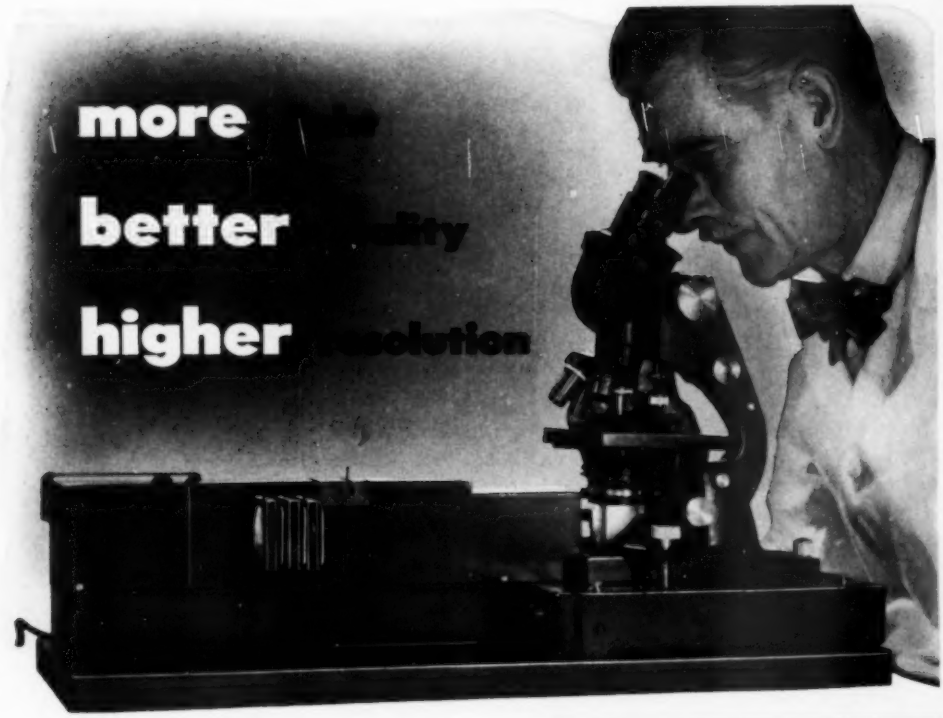

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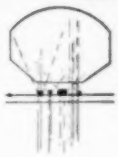

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